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Efectos de la fertilización sobre la composición de macroinvertebrados en un arroyo de montaña tropical

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Effects of Nutrient Enrichment on the Macroinvertebrate Composition in a Tropical Mountain Stream

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A mi padre y abuela por el pasado,
a mi madre y hermanos por el presente,
a John, Alicia y Juan Pablo por el futuro.

Mirar el río hecho de tiempo y agua
y recordar que el tiempo es otro río,
saber que nos perdemos como el río
y que los rostros pasan como el agua.

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Resumen- En un arroyo de montaña andino se estudiaron las respuestas estructurales y funcionales de los macroinvertebrados acuáticos a la adición de nutrientes. Los taxa dominantes fueron: Chironomidae, Ephemeroptera (Baetidae y Leptohyphidae), Amphipoda (Hyalellidae) y Trichoptera (Leptoceridae). El régimen hidrológico fue uno de los principales factores que afectó la distribución de invertebrados a lo largo de la cuenca. Para analizar el efecto del enriquecimiento de nutrientes sobre los cambios en la densidad, la biomasa y la estequiometria en este arroyo, se utilizó un diseño BACI (*Before-After,Control-Impact*). Se encontró que la adición de nutrientes tiene un efecto positivo sobre el incremento de la concentración de la clorofila *a* del biofilm que no se refleja significativamente en la densidad, la biomasa y las relaciones estequiométricas (C:N,C:P,N:P) de los consumidores. También se estudió el efecto de la adición de nutrientes sobre la red trófica por medio de la utilización de las relaciones isotópicas (δ^{15}N y δ^{13}C) en diferentes compartimentos tróficos. Una reducción en δ^{15}N se observó después de la fertilización en el compartimento de los colectores pero no en los depredadores. La señal de δ^{13}C sobre el biofilm presentó una superposición con respecto a la señal de los consumidores primarios. En un experimento *in-situ* utilizando cámaras con biofilm y herbívoros (*Tricorythodes* sp.) la adición de nutrientes presentó un leve incremento en la biomasa de los herbívoros y redujo la biomasa algal en comparación con las cámaras que no presentaban herbívoros. Estos resultados muestran que el exceso de nutrientes se propaga con efectos *botton-up* y *top-down* a través de la red trófica. La eutroficación puede producir cambios en las redes tróficas de los arroyos de alta montaña tropicales.

Palabras clave: Arroyo de montaña andino, enriquecimiento de nutrientes, macroinvertebrados, densidad, biomasa, estequiometria, red trófica, isótopos estables.

Abstract- We studied the structural and functional aquatic macroinvertebrates response to the nutrient enrichment in an Andean mountain stream. The dominant taxa in Tota stream were: Chironomidae, Ephemeroptera (Baetidae and Leptohyphidae), Amphipoda (Hyalellidae), and Trichoptera (Leptoceridae). Hydrological regime was one of the main factors affecting the invertebrate distribution along the basin. To analize the effect of nutrient enrichment on macroinvertebrate density, biomass and stoichiometry in this tropical Andean stream we used a BACI design (Before-After, Control-Impact). We found that the addition of nutrients had a positive impact on the increase of chlorophyll *a* concentration in biofilm but this effect was not significantly reflected in consumer density or biomass and stoichiometric ratios (C:N,C:P,N:P). Also we studied the effect of nutrients in food web structure using isotopic ratios (δ^{15}N and δ^{13}C) in different trophic compartments. A depletion in δ^{15}N was observed after fertilization in collectors compartments but not for top predators. δ^{13}C signal of biofilm had an overlap with primary consumers' signal. In an *in-situ* experiment using chambers with biofilm and grazers (*Tricorythodes* sp.) nutrient addition produced a slight increase in grazer biomass, and reduced algal biomass compared to grazer-free chambers. These results showed that nutrient excess spread bottom-up and top-down effects through the food web. Eutrophication may produce changes in the food web of tropical high-mountain streams.

Keywords: Andean mountain stream, nutrient enrichment, macroinvertebrate, density, biomass, stoichiometry, food web, stable isotopes.

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Chapter 1

General Introduction

1. General Introduction

1.1. Introduction

Rivers in their natural state are the main supply of quality water for a large part of the world. The quantity and quality of water experiences natural variations linked to planetary dynamics (Beckmann *et al.* 2005), and also to global changes. Global predictions indicate that the high latitude river basins may experience an increase in runoff, while others in temperate and tropical zones may see a reduction in the quantity of circulating water therefore the supply for determined uses may be compromised (Nijssen *et al.* 2001).

In any case, the hydrological cycle will be seriously affected. To the alteration of the total runoff must be added its lack of regularity. These aspects are closely linked to the global alterations of the water cycle and the major parts of these alterations are connected to human activities. The expansions of these activities have altered the aquatic systems in an extensive way, modifying the physical characteristics, and the chemical and biological systems. For example, the use of water for irrigation, consumption and other activities represent between 4000-5000 km^3 y^{-1} globally, this is almost 10% of the water circulating through the fluvial watercourses (Sabater *et al.* 2006).

The effects linked to the water flow variations are numerous. The quantity of sediments transported from the fluvial watercourses to the sea has decreased globally by almost 20% (Syvitski *et al.* 2005), and the time of water permanence has increased (Vörösmarty & Sahagian 2000). This time increase of water permanence and greater sedimentation may have important effects on the general character of the river, since the water slows and the river becomes more “lake like” and less “fluvial”; the river has greater hydrologic stability, and the natural variations of the flow become less frequent. In general, the fluvial systems are under the effects of a great variety of impacts (Carpenter *et al.* 2011, Vörösmarty *et al.* 2010), among others, the addition of a growing amount of organic matter. The quantity of nutrients that enter the fluvial systems increases year after year due to inadequate agricultural activities, grazing, fertilization of tree farms, or the direct entrance of waste from urban centers (Maybeck 2003).

The rivers are systems that have the capacity for auto-purification, which generates enormous economic and social benefits. Unfortunately, the volume of contaminants

dumped in the rivers has over taken the capacity of the auto-purification systems (Bernot & Dodds 2005), aggravated by the modification of its geomorphology and the river bank vegetation, such as the changes in ground use.

In this dynamic of the constant addition of nutrients, the small fluvial watercourses are essential in the context of the river basins, since they are key elements in the utilization of these materials (Alexander & Smith 2000), and at the same time may also be strongly affected by them. For example, it has been observed that the concentration of nitrate is greater in lower level brooks than in major rivers, since as the size grows its use as a nutrient by the algae increases (Binkley *et al*. 2004). At this time, little is known as to how these systems assimilate the addition of nutrients without causing appreciable modification in the biological structure (diversity) or in the performance (metabolism).

The major part of the fluvial system, especially the headwater and the final reaches are characterized by a negative net metabolism in that which dominates the processes of respiration before those of production, a consequence of the high quantity and low lability of the organic material that enters these systems (Fisher & Likens 1973). In this case, nutrients may favor the growth of the autotrophic organisms (especially algae) providing high quality food, essential for the trophic chains in systems most heterotrophic (Thorp & Delong 2002). In these systems, the primary production pulses are generally associated with the entrance of resources or limiting factors, whether of light or nutrients (Acuña *et al.* 2004, Sabater *et al.* 2005, Smith & Hollibaugh 1997).

Studies made up to now, have been very enlightening as to the ecosystems answers to the addition of nutrients, mainly N & P. Peterson *et al.* (1993) and Slavik & Peterson (2004) have analyzed the effects caused by the nutrients in the boreal systems, and have determined that they do not only affect the primary producers but also extend to those next to the trophic levels, with changes in the spatial composition and distribution of the aquatic organisms after the arrival of the nutrients. Polis *et al.* (1997) observed that an increase of nutrients and greater hydrologic stability promotes the dominance of the primary producers; this dominance directly favors the herbivores. Sabater *et al.* (2011) described a significant effect of the addition of nutrients in the stoichiometry of the consumers and the biofilm in an oligotrophic, Mediterranean stream. These same authors analyzed the short

term answer in a previous work (Sabater *et al.* 2005) demonstrating that the effect of nutrient additions are made more evident in the increase of the biomass of the autotrophic community and in the metabolic activity, which stimulates an increase in the organic material, which favors the herbivores and the detritivores.

Also, in the oligotrophic fluvial systems based on the external ports, such as river headwaters, the enrichment in nutrients favor the decomposition of organic material, at the moment of stimulation by microbial activity (Suberkropp *et al.* 2010). The effects of the nutrients on the organic detritus extend to the consumers (Cross *et al.* 2005, 2006) even though there is no common answer among the different undertakings.

Studies made by Hillebrand *et al.* (2002), show that fertilization presents a positive relation with the autotrophic and heterotrophic components of the periphyton, which permits the increase of the biomass of the ciliates and the meiofauna. However, the presence of macroinvertebrate, herbivores have a negative effect on the algae biomass which was stimulated by the application of the nutrients (Forrester *et al.* 1999).

In experimental studies it has been demonstrated that the taxonomic composition of algae is more affected by grazing than by the addition of nutrients and that the presence of herbivores and the nutrient enrichment influence the contained nutrients in periphyton (Hilldebrand & Kahlert 2001). Notwithstanding the studies made about the effects of the enrichment of nutrients, there is no uniformity in the answer at the level of biological structure (composition, diversity) and performance (production, decomposition, and metabolism) of the fluvial systems. For example, in fluvial systems which receive extra nutrient support, it is hoped that the biological communities will evolve to a simplification, expressed by a decrease in the biodiversity and the biological connectivity (Figure 1). This lack of a clear answer could be the result, in part, of the temporal hydrological variability of the rivers.

The frequency and intensity of physical (hydrological) events are keys to the processes which occur in the system (Prat 1991). In tropical rivers, since all the system is periodically subjected to perturbation (high flow, low flow, followed by a return to the "ordinary" flow), this combination of events of auto-organization complicates the study of

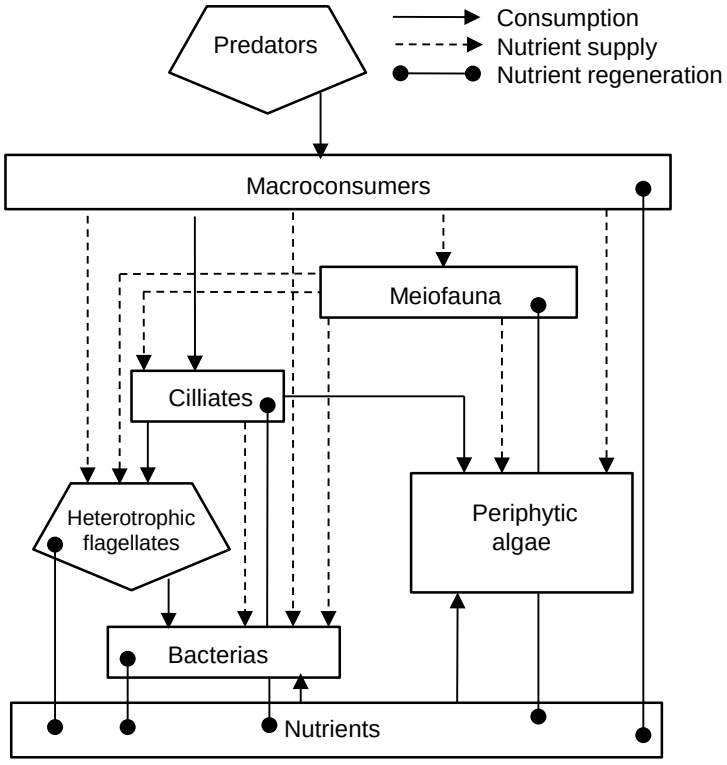

Figure 1. A conceptual diagram of the interaction among the communities of organisms in the aquatic system. Hillebrand, *et al.* (2002).

nutrient effects, which increases its interest and the ecological significance. One way to counter this variability is to analyze these effects on the community at the moment of performance functional changes and not only structural ones.

The results presented by the bibliography have given us an overall view of the theoretical bases applicable to the rivers of the temperate zones. In many cases this is difficult to transfer to tropical rivers due to the differences presented, since the tropical rivers possess a watercourse much longer through the altitudinal, climatic and biogeographical gradients. Lamentably, no studies have been found which classify the tropical rivers in accordance with these characteristics, which has impeded a greater depth of comprehension of these ecosystems (Castro 2005). In the context of global change it is important to be aware, in a tropical climatic environment, the effects of the anthropic pressures, such as the increase of nutrients, on the structure and funtion of the fluvial community.

1.2 Study Site

The Tota stream (5° 35' N - 73° 00' W) is a third order creek, it originates in the eastern mountain range, in the Las Alfombras paramo located in the department of Boyacá (Colombia). The drainage basin covers an area of 150 Km^2.

1.2.2. Geology

According to Pérez & Mariño (1995), the studied zone is of marine origin and chronologically goes from the Cenomanian to the Oligocene. The deposits at the site are basically quaternary of the fluvial lake type and with contributions from the igneous body. At the beginning of the quaternary the volcanic emplacement of acidic rocks in the sector of Iza and its respective hydrothermal action occurred. Near the later Pleistocene there was a facie of lake like fluvial sedimentation that ended with the formation of the new valleys of Iza that were products of the erosion of the Tota and Pesca streams, which empty into the Chiquito river (Buitrago *et al.* 1987). Simultaneously to these events, glaciers were developed which generated the glacier deposits to the south east of Iza. This erosive phase contributed to the degradation and the opening of the igneous body and the surrounding rocks forming cones of volcanic debris and the irregular morphology which can currently be seen. The presence of rocks of varied composition generated an accumulation of soft material that encircles blocks that then become embedded with pieces of resistant rock which generates small movements that become greater in rainy period (Pérez & Mariño 1995).

According to IGAC (1980), the study zone corresponds to a small valley characterized by stable zones of recent softening and the accumulation of materials of coluvial-alluvial origin, where there is no flooding for long periods of time and the drainage is moderate. The ground in this area is described as similar to Ubate, flat and with gradients of 0 to 3%.

The soils correspond to the consociation Ubate principally located in the municipalities of Tibasosa, Sogamoso and Nobsa. These soils originated from clay, are flat with a natural moderate to imperfect drainage. This area is composed principally of the whole of Ubate set (Vertic Eutropept) by 80%. It also presents inclusions of others soils like Nemocón and Chicamocha. The chemical characteristics of this soil are: reaction slightly acidic, cationic

capacity with a high change related to the clay content, high total bases, very high total saturation, very high calcium and magnesium saturations, normal potassium saturations and the phosphorus content is normal to high (IGAC 1980).

1.2.3. Climate

The average temperature registered in the zone is between 10.5 y 11.8°C (Figure 2) and the annual precipitation is 730.5 mm. There are two rainy seasons, April to May (97.6 to 87.4 mm) and October to November (86.1 to 76.7 mm) and two dry seasons, December to January (26.2 to 13.8 mm) and August to September (Figure 3).

1.2.4. Hydrology

The average volume of the Tota river is 0.67 m^3 s^{-1} (average of the values reported between 1965 and 2001). The maximum values are from May to June, with an important peak in October-November and minimum values from December to March (Figure 4).

In the last 20 years there has been noted a decrease of the annual average values of the river volume, possibly as a result of a greater demand for water to use in the irrigation of farms in the area and as a result of climatic changes on a greater scale.

1.2.5. General Physical and Chemical Characteristics of the Water

The conductivity values in the Tota waters are between 27 – 373 µS cm^{-1}, pH has a neutral behavior of 6.67 – 7.93. The nutrient concentrations have ranges of 0.09 – 0.87 mg l^{-1} PRS, 0.1 - 0.5 mg l^{-1} NH_4+, 2.5 - 11.5 mg l^{-1} silica, 0.06 - 0.6 mg l^{-1} NT and 0.05 - 1.4 mg l^{-1} PT (Zapata & Donato 2008).

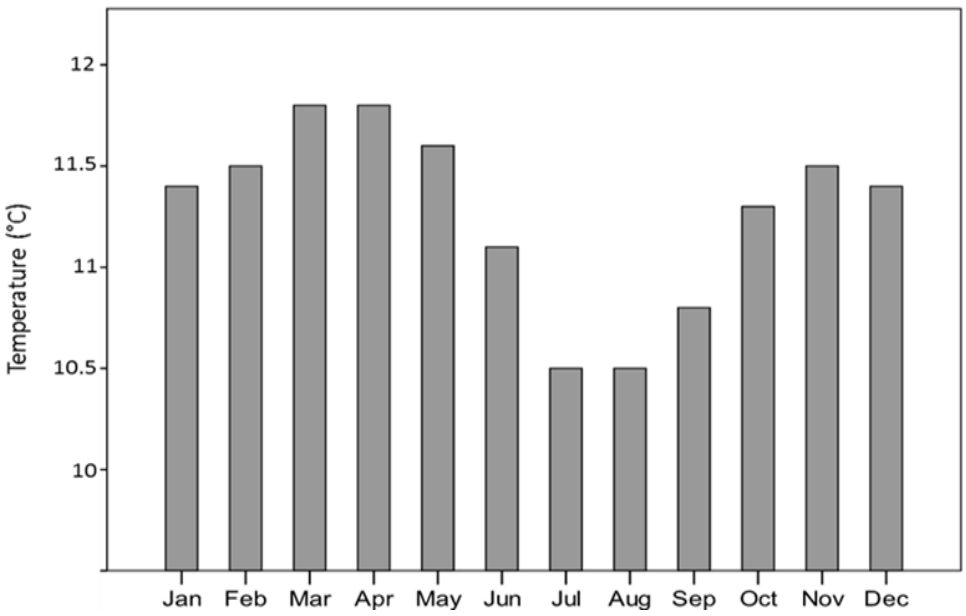

Figure 2. Mean monthly multi-annual values of temperature (°C), registered from 1971 to 1998 at the El Túnel station (IDEAM), located in Cuítiva, Boyacá.

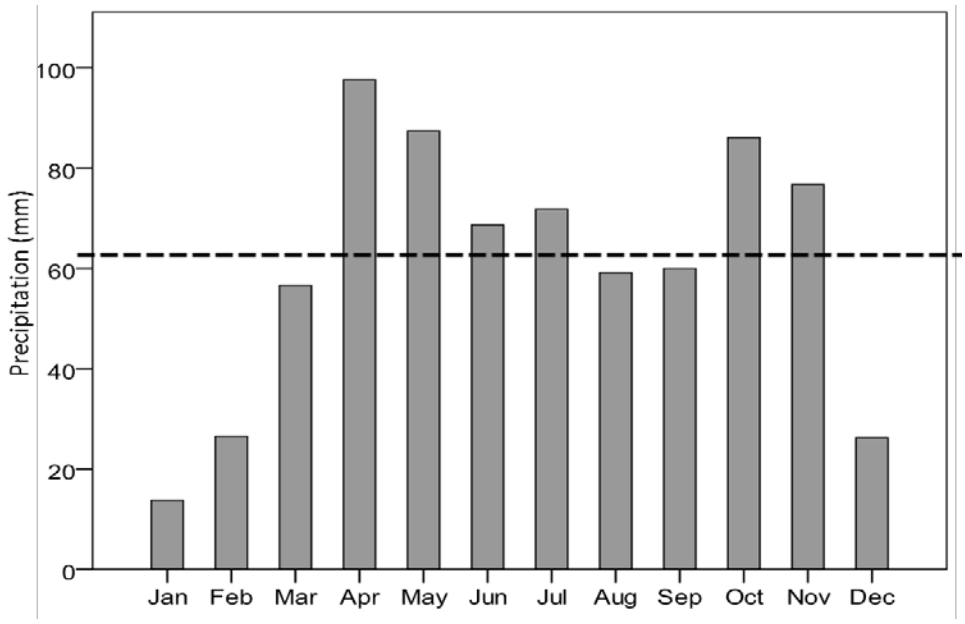

Figure 3. Mean monthly multi-annual values of precipitation (mm), registered from 1971 to 1998 at the El Túnel station (IDEAM), located in Cuítiva, Boyacá. The broken line indicates the average of the values.

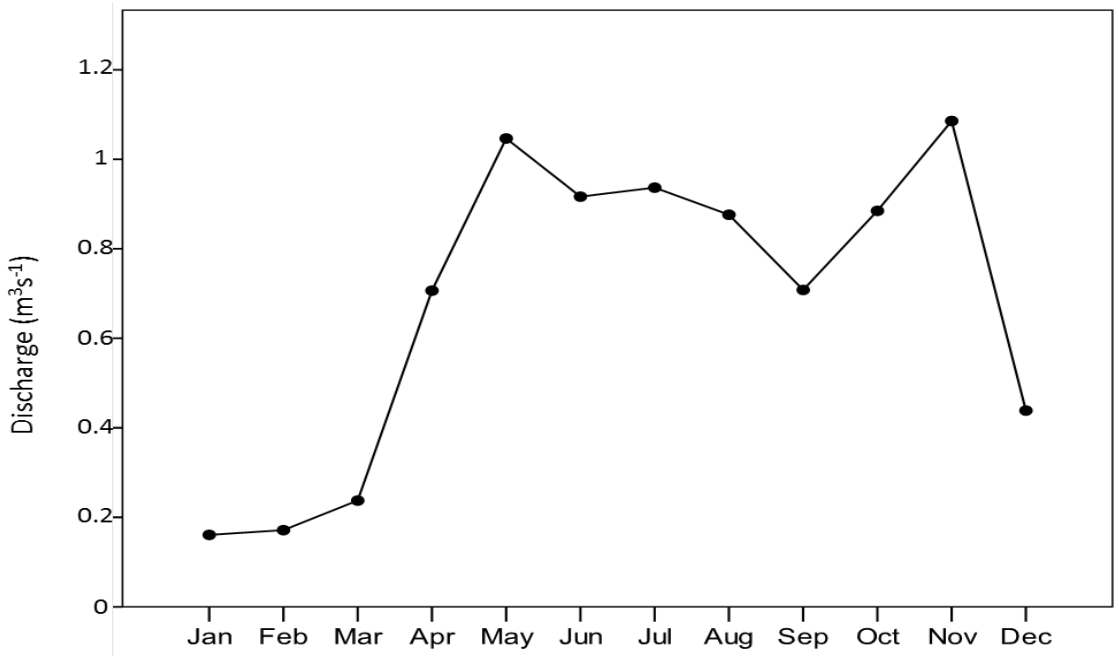

Figure 4. Historical record from1965 to 2001 of the average Tota stream discharge ($m^3 s^{-1}$) (Station La Vega, IDEAM).

1.2.6. Description of the Sample Stations

The high sample zone was taken in the sector of the municipality of Tota (Figure 5). In the mid zone two principal points were established, one in the municipality of Cuítiva named Control (C) and the second point Batán named Impact (I) (Figure 5 and 6). In the low zone the sample point was located in the municipality of Iza (Figure 5).

Four sampling stations were selected along the Tota stream. The streambed was composed by rocks, cobbles, boulders and a few deposits of sand and detritus. At each site, a 50 m reach was selected where were taken: 1) measurements of the descriptive environmental variables of the system, 2) studies of the composition and basic structure of the biological components and 3) estimations of the global measurements of the system.

The following gives the general characteristics of each one of the sampling stations represented in Figure 5

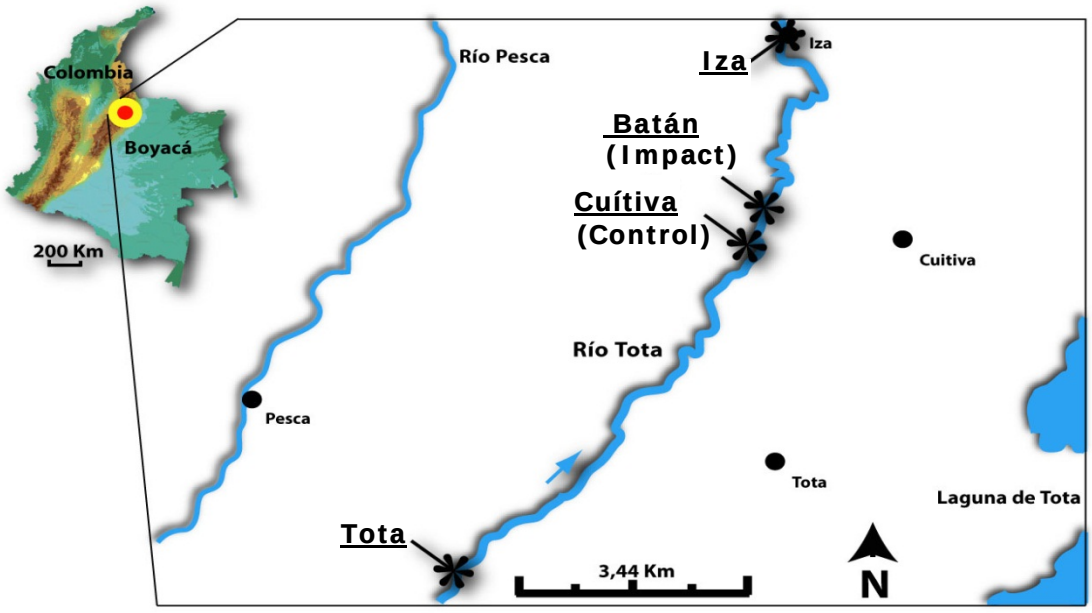

Figure 5. Location of the Tota stream and the sampling stations of the present study.

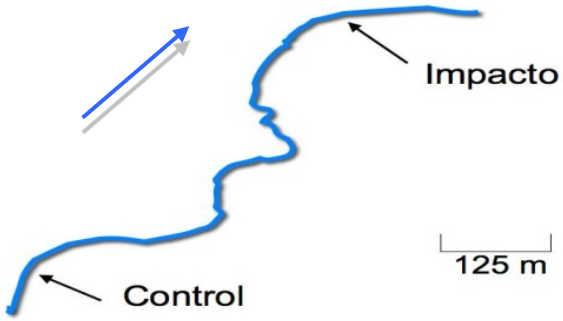

Figure 6. Location of the sampling points where the experiments of fertilization took place. Impact corresponds to the Batán reach (I) point and Control (C) corresponds to the Cuitiva reach.

- **Tota**

Located in the medium high part of the stream basin in the municipality of Tota at 05°33'36'' N and 73°02'45'' W, to 2834 m elevation. It has a low slope with an intensive use, primarily for cattle, but there is also seen native scrub vegetation in different states of development and abundant *Eucaliptus globulus* planted in a scattered form. It represents an area with an almost intact fluvial habitat and the physical chemistry quality of the water is optimum.

- **Cuítiva (Control)**

Located in the municipality of Cuítiva with coordinates of 05°58'15'' N and 72°98'64" W, to 2573 m elevation, corresponds to the medium low part of the stream basin, the bank is composed mainly of willows (*Salix humbodltiana*) and some alders (*Alnus acuminata*) planted but rather scant. Its slope is approximately 3%. In the sector are seen areas with a strong anthropic intervention, primarily used for cattle and in a lesser proportion for cultivation. It represents a fluvial habitat with the presence of nutrients in the water due to human activities, agricultural and ranching in the municipality of Cuítiva.

- **Batán (Impact)**

Located at approximately 695 m down waters from the control reach. It possesses characteristics very similar to the previous reach which is of great importance in establishing the comparison stated in the fertilization experiment. The coordinates of this point are 05º58'61" N and 72º98'43" W and has an altitude of 2567 m elevation.

- **Iza**

Located in the municipality of Iza in the lower part of the stream basin, its coordinates are 05°61'28'' N and 72°98'20'' W, and has an altitude of 2529 m. In this section, the banks of the river have few trees, mainly willows and the slope is similar to that of the Cuítiva station. This section is subjected to the drainage of residual waters from the municipality of Iza. The surroundings correspond to the lower part of the river, where the valley is

characterized by large plains where cattle is raised. It represents an altered fluvial habitat with the presence of nutrients caused by the diverse activities in the Municipality of Iza.

1.3. Problem Formulation

The primary production in fluvial headwater systems, are in part, determined by the availability of nutrients. The nutrients also favor the development and activity of the microbial biofilm (algae, bacteria and fungi) in the allochthonous organic matter proceeding from the contributions of the riparian forest. The authochthonous and allochthonous organic mattter are basal resources in the trophic fluvial food web. So, what is the effect of a controlled addition of nutrients on the community of macroinvertebrates consumers in a headwater tropical stream?

1.4. Hypothesis

By increasing the availability of nutrients in a reach of the Tota stream, where these are limited, the quality of the basal resources (algae and detritus) will also increase. The greater quality will favor consumers with an increase in the density and biomass. These changes could transfer to the stoichiometric composition of the resources and the consumers, a greater availability of nutrients, a greater proportion of N and P. Some consumers may change diets in favor of a greater proportion of base resources, especially algae, and change the trophic structure in the fertilized reach.

1.5. Objectives

1.5.1. General Objectives

Know the organization in the space and time of the community of macroinvertebrates in the Tota stream.

Determine the effect of addition of nutrients on the communities of macroinvertebrates in a tropical mountain creek.

1.5.2. Specific Objectives

- **Chapter 2**

- Characterize throughout the spatial and seasonal fluctuations, the community of macroinvertebrates of the Tota stream with the study of the relations between the density and the environmental parameters of the stream.

- **Chapter 3**

- Establish the effect of fertilization on the structure of the aquatic system with emphasis on the community of macroinvertebrates, studying in the experimental reaches the changes in the density and biomass.

- Analyze the effects of the addition of nutrients on the basal compartments and the consumers using the elemental composition of C, N, P and its stoichiometric ratios.

- Determine the effect of the fertilization on the stoichiometric balances between consumers and their resources.

- **Chapter 4**

- Describe the trophic relations in the two reaches of the Tota stream through the analysis of stable isotopes $\delta^{13}C$ and $\delta^{15}N$.

- Establish the effect of the nutrients addition on the trophic relations of the different trophic compartments of the stream through the use of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ and the analysis of the gut contents of the macroinvertebrate consumers.

- **Chapter 5**

- Study the effect of fertilization on the increase in the biomass of the nymph organisms of the *Tricorythodes* sp, the primary production and the consumption of algae.

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Chapter 2

Preliminary Studies on Macroinvertebrate Assemblage Distribution in a Neotropical Andean Mountain Stream (Tota, Colombia)

2. Preliminary Studies on Macroinvertebrate Assemblage Distribution in a Neotropical Andean Mountain Stream (Tota, Colombia)

2.1. Abstract

Seasonal and spatial fluctuations in water physicochemistry and density of macroinvertebrates were examined in a Neotropical high mountain stream. A total of 75 samples were collected in three reaches in five different months. In each reach we sampled rocks, sand, leaf litter, macrophytes, and stream margins . We identified 42 families of macroinvertebrates. The dominant taxa were Chironomidae, Ephemeroptera (Baetidae and Leptohyphidae), Amphipoda (Hyalellidae), and Trichoptera (Leptoceridae). The ANOSIM test ($p<0.005$) detected small differences between sites and temporal differences between periods of low and high flow. Pair-wise comparison between substrates showed highest differences between sand and the other habitats. The RDA analysis showed that temperature and discharge significantly influenced the invertebrate community structure. Hydrological regime was one of the main factors affecting the invertebrate distribution along the basin. These results are important to the ecology and conservation of Andean high mountain streams.

Keywords: Hydrological regime, habitats, reaches, trophic strategies, Neotropical stream.

2.2. Introduction

The physical and hydrological characteristics of a stream modulate the ecological processes and determine the distribution of the biological communities that live in it (Allan & Castillo 2007). Most tropical regions experience important seasonal differences in rainfall, alternating between unstable and more stable periods (Lewis 2008). In the case of the Andean high mountain rivers, slope is responsible for regulating discharge, velocity and, in general, the river energy that becomes a controller of the physical and biological

properties in the system (Donato & Galvis 2008). Habitat stability has a primary influence in the life history of the organisms and population dynamics in these tropical mountain streams (Jacobsen *et al.* 2008).

The importance of altitudinal gradient in the macroinvertebrate composition in the Andean streams has been studied by several authors (Jacobsen & Encalada 1998, Jacobsen 2003, 2004, Acosta & Prat 2010). At local spatial scale benthic invertebrates are strongly influenced by the hydrological regime, which, in turn, depending on the riverbed composition (Flecker & Feifarek 1994, Jacobsen 2005, Acuña *et al.* 2005), and by the ecological conditions such as spatial habitat stability and heterogeneity. Patches with different grain size constitute different functional habitats (Harper *et al.* 1992) and it is possible to identify species or assemblages associated to these habitats, where invertebrates can complete their life cycle (move, rest, find refuge and feed) (Minshall 1984, Tomanova & Usseglio-Polatera 2007). Refuge availability is of great importance for the species because it protects them from being dislodged by a disturbance such as the seasonal hydrological changes characteristic of temperate streams (Winterbottom *et al.* 1997, Lancaster 2000).

In regions where rainfall is highly seasonal, Neotropical streams, particularly those of small size, may be subject to unpredictable and severe disturbances (Flecker & Feifarek 1994). Previous studies in tropical high mountain streams of Colombia have pointed out the importance of hydrological variables on the structure and stability of the macroinvertebrate assemblages (Rodríguez *et al.* 2006; Amaya 2008, Castro & Donato 2008, Rincon & Castro 2008). However, little is known about the habitat distribution of these invertebrates and the factors that determine it.

Here we examine seasonal and spatial fluctuations in invertebrate density along a Neotropical high mountain stream. Our main objective was to determine whether differences exist in spatial (habitats) distribution in community structure and which variables drive this distribution. Furthermore, we wanted to discern whether hydrological differences between rainy and dry seasons act as a disturbance effect on invertebrates. Finally, we compared trophic strategies for the different habitats during the different seasons studied. We hypothesized that invertebrate assemblage structures will differ between habitats, with invertebrates being most abundant in the more stable habitats.

2.3. Methods

The sampling was carried out during the low discharge (December 2006, April 2007, February 2008) and high discharge periods (August and October 2007) in Tota, Cuitiva and Iza reaches. In each reach we sampled the following habitats: rocks, sand, leaf litter, macrophytes and stream margins. Due to the absence of substrates in some sampling dates as a consequence of hydrological variations in the stream, two samples were collected from rocks and leaf litter and one for the rest of the habitats for each reach and sampling date. The samples of rock habitat were taken using a Surber of 900 cm^2, while leaf litter, macrophytes and stream margins were sampled with a Surber of 400 cm^2. Sand was sampled with a 54 cm^2 core. Each device was selected in function of the characteristics of each substrate. Samples were cleaned with stream water and filtered through different sieves, (the smallest, 0.5 mm mesh size) and preserved in alcohol (70 %). Macroinvertebrates were determined according to family level and the Functional Feeding Groups (FFGs) were identified according to Merrit & Cummins (1996), Wallace & Webster (1996) and Rivera *et al.* (2008). Density results were expressed as individuals per m^2, to standardize for all substrates.

Water temperature, dissolved oxygen, conductivity and pH were measured *in situ* (YSI multiparameter, model 5563-10 MPS). Water velocity was measured *in situ* using a Global digital flow meter and discharge (Q). The concentrations of NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} were determined following APHA methods (1998) with filtered water (Brand GF/F).

Taxa abundances were square roots transformed prior to analysis and a preliminary similarity analysis (Bray-Curtis similarity index, Bray & Curtis 1957) was conducted to examine the variation of the macroinvertebrate assemblages.

Afterwards, a hierarchical clustering was used to recognise the different groups of the macroinvertebrate assemblage. ANOSIM (analysis of similarities) was used to analyse whether assemblages differed between groups of samples, defined by habitat type (n= 30 for rocks and leaf litter, n= 15 for the rest of substrates) or sampling time (n=42 in low discharge, n=30 in high discharge) for all three reaches. The SIMPER (similarity percentages) analysis identified the taxa responsible for most of the similarity and dissimilarity within and among groups. The analyses were performed with PRIMER v. 5

(Clarke & Gorley 2001). Additionally, we carried out a redundancy analysis (RDA), in which species data were constrained by environmental variables. Physicochemistry variables, except pH, were transformed to reduce skewed distributions. The RDA ordination assumes a linear combination of the species along the environmental gradients preserving the Euclidean distances. The maximum length of the gradient (2.76) obtained with a DCA indicated that linear methods were appropriate. The RDA analysis was performed with CANOCO software version 4.5 (ter Braak & Smilauer 1998).

2.4. Results

2.4.1. Environmental Parameters

Table 1 shows the mean values of the physicochemistry variables measured in the Tota stream. Months with low flow levels corresponded to December, April and February, while months with high flow were August and October. During the high flow, temperature presented a longitudinal gradient from 12.2 °C to 13.6°C, while in the dry months it was between 12.4°C and 17.5ºC. The oxygen concentration (9.0 mg L^{-1}) was highest in Iza during high flow and lowest in the same station for the dry period (7.0 mg L^{-1}). In low flow conditions, conductivity values increased in the downstream. In general, the pH had no significant variations. PO_4^{3-} concentration was lower during high water periods. As expected, the lowest NH_4^+ concentration was found in the Tota reach (0.08 mg NH_4^+ L^{-1}). $NO_3^-$and NO_2^- concentrations were always below the detection limit (0.01 mg L^{-1}).

Table 1. Average values of the physicochemical characteristics for the three reaches in the different flow periods.

	TOTA		CUITIVA		IZA	
Parameter	**Wet season**	**Dry season**	**Wet season**	**Dry season**	**Wet season**	**Dry season**
Discharge (m^3s^{-1})	1.4	0.2	1.2	0.1	1.8	0.2
*D. O. (mg l^{-1})	7.6	7. 9	8.7	7.5	9.0	7.0
pH	7. 3	7. 4	7.3	7.3	7.5	7.3
Temperature (°C)	12.2	12.4	12.8	15.6	13.6	17.5
NH_4^+ (mg l^{-1})	0.17	0.08	0.14	0.11	0.16	0.13
PO_4^{3-} (mg l^{-1})	0.05	0.11	0.05	0.11	0.07	0.25
Conductivity (µs cm^{-1})	55.6	48.9	64.8	131.5	84.5	96.2

*D.O. Dissolved Oxygen

2.4.2. Macroinvertebrate Assemblage

We identified a total of 42 families and family richness was highest in Iza (Table 2). Dominant taxa were Chironomidae, Ephemeroptera (Baetidae and Leptohyphidae), Amphipoda (Hyalellidae), and Trichoptera (Leptoceridae). The ANOSIM test found significant differences, ($p = 0.001$) between sites. In Tota, Chironomidae, Baetidae, Leptohyphidae and Leptoceridae contributed 70 % of the total density in this site. In Cuitiva the same families were important, but Chironomidae had a higher abundance than in Tota. On other hand, in Iza two other families were also important (Hyalellidae and Elmidae). The highest dissimilarity (average dissimilarity 79.2) was found between Tota and Iza and was related to higher abundances of Trichoptera, Amphipoda and Basommatophora downstream.

Temporal differences (ANOSIM, $p<0.05$) were observed between periods with high flow (August and October) and low flow (December, April and February). In general, the total number of families was lower during the rainy season when the density of Baetidae increases while the density of Chironomidae decreases (Figure 1). However, these changes were not as pronounced in the headwater (Tota reach).

There were no significant differences in functional feeding group and density between sites and seasons. The density of scrapers and collector-filterers were similar (30% each), while collector-gatherers and shredders accounted for 15% each.

2.4.3. Composition of the assemblages in the different habitats

The highest difference in the structure of the macroinvertebrate assemblages for all samplings was between sand and the other habitats (R values >0.49, ANOSIM, Table 3). In sand, the most abundant families were Chironomidae, Oligochaeta and Elmidae, which contributed nearly 85 % of the total density. Baetidae was the most abundant family in the rest of the habitats (except leaf litter), accompanied by Chironomidae, Hydroptilidae,

Table 2. Mean and Standard Deviation (SD) values of macroinvertebrate density (Org m^{-2}) in the three sampling sites.

			Tota		Cuítiva		Iza	
Clase	Order	Family	MEAN	SD	MEAN	SD	MEAN	SD
Hydrozoa	Hydroidea	INDETERMINATED	8.85	40.79	0	0	0.46	2.27
Turbellaria	Tricladida	Dugesiidae	9.09	30.9	0	0	56.93	81.89
Oligochaeta		INDETERMINATED	811.01	2135.06	735.51	2750.6	806.13	2784.16
Hirudinea		INDETERMINATED	0.23	1.13	0.52	2.55	397.16	1729.07
Malacostraca	Amphipoda	Hyalellidae	129.89	267.14	816.79	1512.26	2736.91	6250.52
Aracnida	Acarina	Hydracarina	93.04	154.78	54.44	84.86	12.27	21.37
Insecta		Entomobryidae	1.56	5.61	0.23	1.13	0.42	2.04
	Collembolla	Isotomidae	2.63	10.44	15.71	61.15	3.47	11.68
		Poduridae	0	0	0.52	2.55	0	0
		Sminthuridae	0	0	0.23	1.13	0	0
	Plecoptera	Perlidae	21.64	44.18	0	0	2.08	10.21
	Ephemeroptera	Baetidae	923.72	1128.48	1984.15	2039.18	2750.02	2962.27
		Leptohyphidae	2058.46	4584.57	1544.73	1689.51	261.64	313.32
		Leptophlebiidae	105.9	175.25	106.75	177.95	174.41	400.1
	Odonata	Aeshnidae	0	0	3.13	15.31	17.5	61.59
		Coenogrionidae	0	0	0	0	1.35	5.26
		Libellulidae	0	0	0	0	0.31	1.12
	Hemiptera	Corixidae	0	0	0	0	0.21	1.02
		Notonectidae	0	0	0	0	2.29	6.47
	Trichoptera	Glossosomatidae	0	0	106.6	212.87	705.98	2139.96
		Helicopsychidae	84.89	200.76	1.04	5.1	0	0
		Hydrobiosidae	49.28	89.33	10.52	16.18	1.06	3.43
		Hydroptilidae	806.54	1638.95	684.7	1033.26	310.17	530.56
		Leptoceridae	1217.62	1933.51	923.43	1293.92	1017.26	2305.03
		Odontoceridae	1.04	5.1	0	0	0	0
		Policentropodidae	1.04	5.1	3.13	15.31	0.46	2.27
		Xiphocentronidae	0.55	2.69	134.9	450.08	83.84	285.06
	Lepidoptera	INDETERMINATED	1.64	5.9	1.04	5.1	5.89	17.32
	Coleoptera	Curculionidae	0	0	0.52	2.55	0	0
		Dysticidae	0.52	2.55	0	0	27.6	95.12
		Elmidae	715.09	2099.8	480.13	588.98	383.72	576.8
		Gyrinidae	0	0	0	0	0.1	0.51
		Helodidae	0.23	1.13	0	0	0	0
		Psephenidae	0	0	5.67	10.96	145.4	551.47
		Staphilinidae	0.78	2.87	2.14	6.24	1.1	5.37
		INDETERMINATED	1.1	5.37	0	0	0	0
	Diptera	Blephariceridae	10.42	38.72	0	0	0.55	2.69
		Ceratopogonidae	240.74	758.76	76.49	167.87	76.19	169.24
		Chaboridae	0	0	0.52	2.55	0.52	2.55
		Chironomidae	1990.91	4719.3	2822.2	5655.66	2877.99	5650.73
		Dixidae	0	0	7.7	37.73	7.7	37.73
		Empididae	20.39	33.36	24.5	41.35	11.42	25.48
		Psychodidae	9.26	37.81	1.79	5.65	0.23	1.13
		Simuliidae	423.07	650.79	296.53	468.65	431.18	782.06
		Tipulidae	12.36	38.1	9.79	38.12	3.21	9.38
		INDETERMINATED	0	0	7.7	37.73	0	0
Bivalvia	Veneroida	Sphaeriidae	38.74	133.22	35.83	82.74	105.39	316.8
Gastropoda	Basommatophora	Physidae	9.03	37.84	62.38	204.32	834.33	2317.51
		INDETERMINATED	0	0	0.52	2.55	0	0
Family richness			34		36		40	

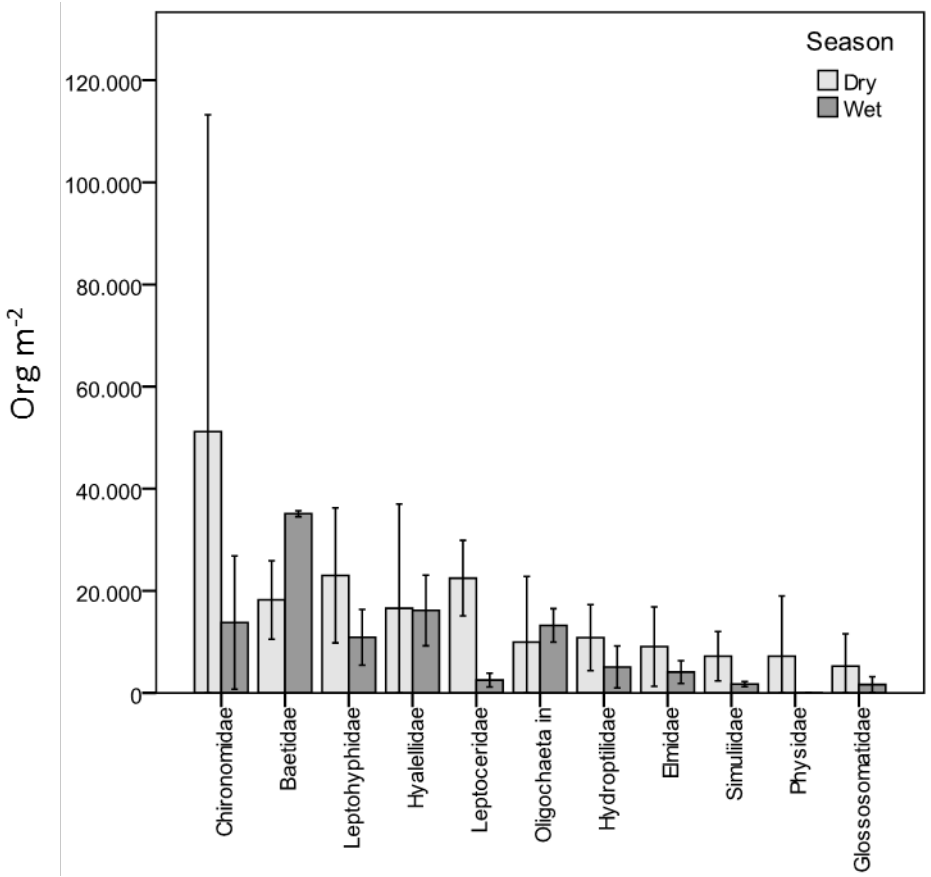

Figure 1. Mean Density of the different macroinvertebrate families during the wet season and dry season in the Tota stream, including all substrates.

Table 3. R value for each pair comparison. Values close to unity were indicative of complete differences between the groups, values close to zero implied small differences. Only comparisons with significance level p≤ 0.001 are indicated.

	Rocks	**Sand**	**Margins**	**Litter**	**Macrophytes**
Rocks					
Sand	0.585				
Margins	0.280	0.494			
Litter	0.251	0.513	0.040		
Macrophytes	0.278	0.525	0.030	0.071	

Leptoceridae and Leptohyphidae in rocks and macrophytes. In the leaf litter, Chironomidae appeared as the most abundant family with 25%, followed by Baetidae with 19%, while Simuliidae presented a lower density (approximately 11%), Hyalellidae was found mainly on stream margins (13.5%). The highest differences were found in February when the density in sand reached 80000 ind m^{-2}. Similar results were observed regarding the contribution of the feeding groups in each substrate. Maximum differences were found between rocks (40% of the density was for scrapers) and fine sands (60% of the density for collectors).

2.4.4. Relationships between macroinvertebrate assemblages and environmental variables

The RDA analysis showed that temperature and discharge significantly influenced the invertebrate community structure (Figure 2). The two first axes explained 71.4% of the variance in invertebrate density ordination. The first axis reflected the distribution of sites along a gradient of temperature. The abundance of the Physidae, Chironomidae and Hyalellidae families were closely associated with the warmest waters at downstream sites, mainly when flow was low. The second axis showed a discharge gradient. The October sampling was related to higher flows. Baetidae is the family found with highest density in the rainy period. The rest of the taxa were related to lower flow conditions.

2.5. Discussion

The hydrological regime in the Tota stream was one of the main factors affecting the invertebrate distribution throughout the study period. The high flow period was characterized by lower water temperature, lower density of invertebrates and the most abundant family was Baetidae. This community composition changed to one in which Diptera, mainly Chironomidae, were more abundant during the dry period. This dry period was characterized by low flow, higher water temperature, lower oxygen and PO_4^{3-} concentrations in all stations. Temporal variability of flow produced changes in macroinvertebrate assemblage structure modifying the total density and the relative abundance of some of the taxa. Similar results in tropical streams were obtained by

Turcotte & Harper (1982), Ramírez *et al.* (1998, 2006) and Jacobsen *et al.* (2008). Moreover, Flecker & Feirfarek (1994) and Rincón & Cressa (2000) have described reductions in invertebrate densities during periods of hydrological extremes in two streams of the Venezuelan Andes. The short stability of these Andean streams during the dry season determines a community dominated by organisms with fast growth, which is essential for rapid recovery (Jacobsen & Encalada 1998). Depending on the length of time elapsed since the last disturbance and the magnitude of the event, the community will present more or less variability between sampling dates and sites.

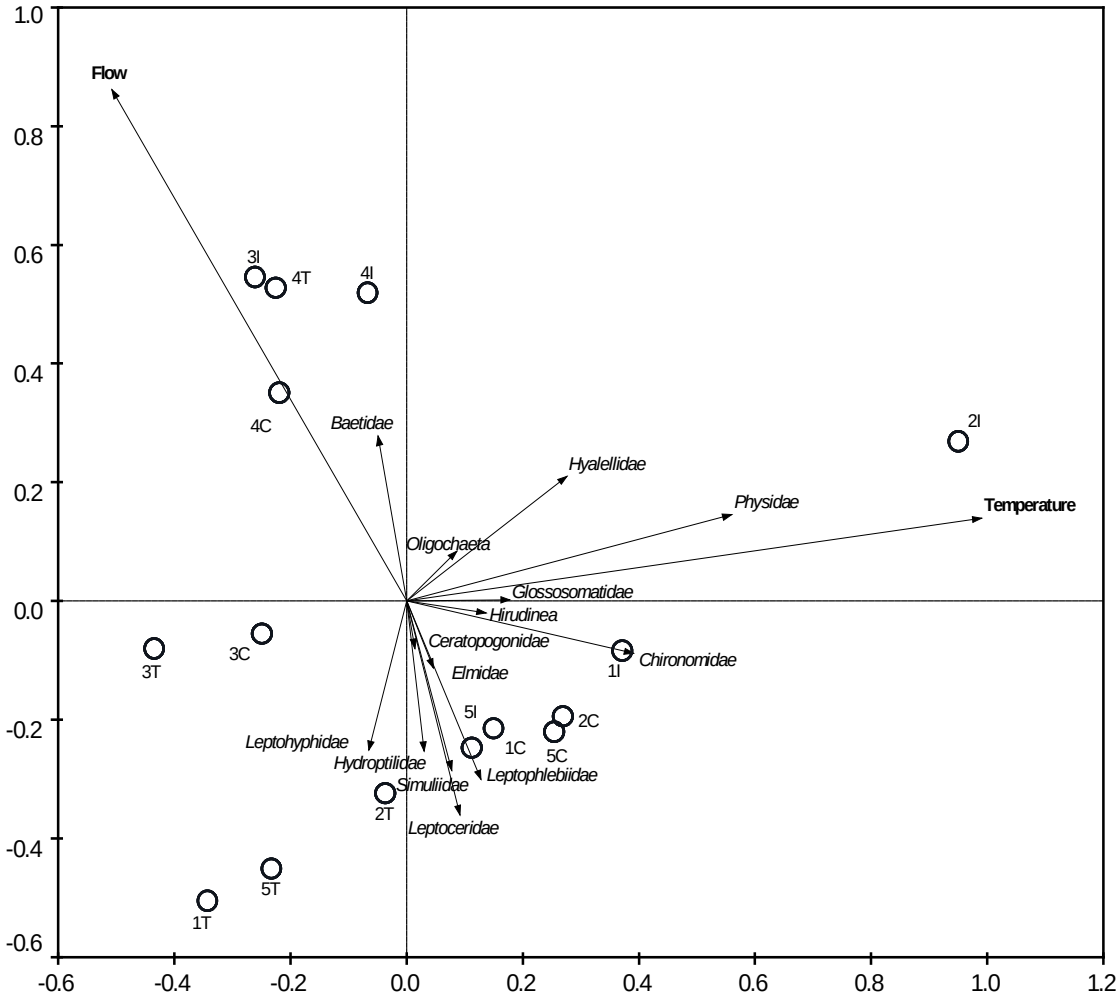

Figure 2. Ordination of sampling sites and invertebrate taxa based on RDA analysis in Tota stream. T (Tota site), C (Cuitiva), I (Iza); sampling periods: 1 (December 2006), 2 (April 2007), 3 (August 2007), 4 (October 2007), 5 (February 2008).

Invertebrate community structure is quite different along the river but the differences were greater between headwaters and the downstream site. The Hyalellidae and Physidae families were more abundant in Iza than in the upstream sites. This seems to be an effect of the higher concentration of nutrients (Zapata & Donato 2005) and availability of light that increase primary productivity and produce a change in the quantity and quality of resources (Donato *et al.* 2010).

The major feeding strategies in the stream were scrapers and collector-filterers with no significant differences between sites. Previous studies have reported the abundance of biofilm in this stream (Martinez & Donato 2003) and its importance for grazers (Donato *et al*. 2010). Covich (1988) and Tomanova *et al.* (2006) have pointed out the high trophic flexibility of the invertebrates in tropical fluvial systems that contributes to an increase in survival ability and facilitates colonization.

Significant differences were found between the invertebrate composition of sand and the others substrates studied. Chironomidae, Oligochaeta and Elmidae contributed with nearly 85 % of the total density in sand. Finer substrates allow animals to penetrate into the sediment for feeding and protection (Malmqvist 2002) and, during stable flows, sand patches are depositional reservoirs for detritus that may be densely colonized by bacteria and fungi, which increase its nutritional value (Gaudes *et al*. 2009). Furthermore, sand substrates are highly susceptible to scouring after floods (Townsend 1989) and the taxa that live in this habitat tend to have traits that make them resilient to floods, such as fast growth and use of habitat refugia (Townsend & Hildrew 1994, Gaudes *et al*. 2010).

Macroinvertebrate fauna in the Tota stream responded to the hydrological regime, which guarantees recolonization after spates. The altitudinal gradient in temperature, particularly in dry period, and the temporal variability in discharge are the main factors that explain the changes in macroinvertebrate assemblages.

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Chapter 3

Nutrient Addition Effects in a Tropical Mountain Stream (Tota, Colombia): Changes in Density, Biomass and Stoichiometry

3. Nutrient Addition Effects in a Tropical Mountain Stream (Tota, Colombia): Changes in Density, Biomass and Stoichiometry

3.1. Abstract

To define the effect of nutrient enrichment on changes in biomass and stoichiometry in a tropical Andean stream, we used a BACI design to analyze its effects. The addition of nutrients had a positive impact on the increase of chlorophyll *a* concentration, but this effect was not significantly reflected in consumer density or biomass. Changes between high and low discharge periods could mask other effects, as with the effects of nutrient addition in this study. The statistical analysis of density and biomass according to Functional Feeding Groups (FFG) presented no significant differences, but a slight increment in density and biomass of collector-gatherers was evident, represented mainly by *Americobaetis* sp., *Camelobaetidus* sp. and *Tharulodes* sp., which are species that may take advantage of detritus and periphyton availability in the impact reach after enrichment. The C:N, C:P and N:P values found in Tota for stream collectors were in the range than those reported by other authors, while fish had higher CP and NP values showing a limitation of P for top predators in Tota. The stoichiometric molar ratios showed no significant changes in relation to the experiment. A t-test of the CNP ratios for some collector-gatherer species showed that only *Tricorythodes* sp. had a significant decrease in C:N ratio in impact reach after nutrient enrichment.

Keywords: nutrient enrichment, biomass, density, stoichiometric ratios, BACI design.

3.2. Introduction

Nutrient enrichment has a global impact on aquatic ecosystems. Human activities are the main source of nutrients entering aquatic systems that drive community changes in freshwater and coastal marine ecosystems (Smith & Schinder 2009). Nutrient enrichment affects structural and functional aspects of stream ecosystems, including algal production, decomposition of organic matter and consumer abundances. In this way, the effects can extend throughout the aquatic food webs (Smith *et al*. 1999). In rivers that depend on

autochthonous primary production, nutrient enrichment has been shown to increase biomass at the base of the food web (Peterson *et al.* 1993; Slavik *&* Peterson 2004). In detritus-based systems, nutrients may increase microbial biomass and the rate of leaf litter decomposition (Gulis & Suberkropp 2003, Ferreira *et al.* 2006) and consequently, detritus becomes a better food source for detritivores. Effects of enhanced nutrients on detritus may extend to the consumers, both in their community composition and productivity (Cross *et al*. 2005a, 2007). The response of the biological communities to the nutrient depends on environmental characteristics that may either favour or constrain the effect of enrichment (Hill *et al.* 2009). Complementary analysis of structural and functional processes could be a more robust approach to assess nutrient effects.

The analysis of variation in elementary composition of different trophic compartments (algae, organic matter, benthic macroinvertebrates) and their relation to environmental factors that influence this variation could also improve our understanding of lotic ecosystems (Frost *et al.* 2002). Nutrient uptake is expected to increase with increasing rates of metabolism due to assimilatory demand. Nutrient addition could favor metabolism mainly in systems where nutrients are limited. Small order streams play an important role at the watershed scale since the proportion of surface area accounted for by them with respect to the total drainage network is high (Tockner & Stanford 2002). In these systems, the benthic compartment becomes an important site for uptake, transformation and recycling of essential elements, and contributes significantly to ecosystem functions, such as nutrient cycling and the whole-ecosystem metabolism (Cross *et al.* 2005a).

Ecological stoichiometry is defined as the balance between multiple chemical substances in ecological interactions and processes (Sterner & Elser 2002), and it deals mainly with the mass balance of carbon (C), nitrogen (N) and phosphorus (P) (Cross *et al.* 2005b). The primary focus is on N and P as essential nutrients, often related to C as an energy source (Persson *et al*. 2010). Enrichment significantly increased the nutrient content of food resources (Cross *et al.* 2003, Sabater *et al.* 2011).

Homeostasis is the resistance to change of consumer body stoichiometric composition in response to the chemical composition of the consumer´s food (Persson *et al.* 2010). Organisms that maintain constant stoichiometry regardless of fluctuations in resources are considered strictly homeostatic, while those whose composition varies in direct proportion

to changes in their resources are non-homeostatic (Sterner & Elser 2002). Generally speaking, autotrophs exhibit flexibility in their composition (Sterner *et al.* 1998), while heterotrophs are confined to a constant (strictly homeostatic) body composition (Persson *et al*. 2010). However, Cross *et al.* (2003) observed that P content of some invertebrates increased due to nutrient enrichment, as did Sabater *et al.* (2011), who also described changes in N content in some invertebrate species after long-term enrichment, both studies suggesting deviation from strict homeostasis.

Imbalance of C:N, C:P or N:P between consumers and their sources can strongly constrain consumer growth and reproduction (Frost *et al.* 2005) and may affect the structure of food webs and constrain or alter key ecosystem processes (Cross *et al.* 2005b). While the P content of vertebrate consumers is largely a function of structural biomass, in invertebrate consumers it is found in metabolic biomass (Vanni *et al*. 2002, Small & Pringle 2010). Ribosomal RNA and other intra-cellular pools of P may vary based on dietary availability of P. Growth efficiency in animals increases with increased food nutrient content (Sterner & Elser 2002). The Tota stream is characterized by low conductivity values and nitrogen limitation (Castro & Donato 2008a), making it an interesting place in which to perform enrichment experiments. The aim of this work is to analyze the effects of nutrient addition in the basal compartments and consumers in the Tota stream, based on: i) changes in chlorophyll of the benthic biofilm and density and biomass of macroinvertebrates, along with ii) changes in the C, N, P, composition and its stoichiometric relations, in order to define balances between consumers and resources. We have proposed three hypotheses: 1) higher nutrient availability will favour primary production and higher chlorophyll concentration will be expected; 2) nutrient addition will increase the content of N and P in the biofilm and debris and can be shown in lower C: N and C: P molar ratios. This higher quantity and quality of resources will be translated into higher biomass of consumers, mainly grazers and collectors; 3) there will be a reduction of stoichiometric differences between resources and consumers. Nevertheless, the consumers will probably retain their composition due to their ability to be homeostatic.

3.3. Methods

3.3.1. Experimental Design

The two study reaches were located in the Tota stream, within the area of the municipality of Cuítiva in the department of Boyacá (Colombia).

In order to define the effect of nutrient enrichment on conservation of the stream, we chose two 50 m reaches that were geo-morphologically and hydrologically similar: Control (C) and Impact (I). Both reaches were studied for 13 months prior to the enrichment. The C reach was located 700 m upstream from the I reach, where the nutrients were added into a 500 L tank. The continuous addition of nutrients was performed using a drop system over a 10-month period. Two commercial grain fertilizers (Nitron 26 (26-0-0) and Abocol (NPK) (10-30-10)) were diluted in the tank in order to at least double the average basal (natural) phosphate and ammonium concentrations in the stream. Nutrient addition was adjusted bi-weekly following the natural in-stream nutrient dynamics, and natural N:P proportions were maintained as well.

3.3.2. Hydrological, Physical and Chemical Variables

Measurements of hydrological, physical and chemical variables were taken bi-monthly. Current velocity and flow (Q) were taken with a global digital flow-meter. Temperature (°C) and dissolved oxygen (mg l^{-1} O_2) were registered with a HACH LDO HQ30d oxygen sensor. Conductivity (µS cm^{-1}) was measured with a YSI model 556 MPS multi-parametric probe. The pH was measured with a SCHOTT pH 11/SET sensor. The ammonium (µg l^{-1} NH_4^+), nitrate, nitrite and phosphate (µg l^{-1} PO_4^{3-}) were all measured by following the techniques described by APHA-AWWA-WEF (2005).

3.3.3. Biological Sampling

- **Chlorophyll *a* in biofilm**

Chlorophyll *a* was measured in the biofilm collected from 1 cm^2 ceramic tiles glued onto rock slabs that were kept at a depth of 10 to 20 cm in riffle zones. It was then analyzed according to standard methods (APHA-AWWA-WEF 2005).

- **Invertebrate Density and Biomass**

Three samples of each substrate (rock and sand) were taken in the two reaches on a bi-monthly basis, before and after the nutrient addition. Invertebrates were sampled directly on the cobbles by using a Surber sampler with a 900 cm^2 surface area and a 500 µm net mesh size, and the sandy substrate was sampled with a 54 cm^2 core. Samples were cleaned with river water and filtered through different sieves, the smallest one being of 0.5 mm mesh. The samples were preserved in alcohol (70%) and the invertebrates were sorted under a dissecting microscope and identified by genus as far as possible.

Biomass values (Dry Mass, DM mg m^{-2}) were obtained by measuring the total body length of organisms and length and width of the cephalic capsule for each animal sampled. The results were fitted to the Burgherr & Meyer (1997) equation: $DM = a*L^b$, where *a* and *b* were the regression constants, DM dry mass (mg) and L total body length (mm). For the gastropods, we used the Eckblad (1971) equation. For Oligochaeta, the size of each animal was assimilated to a cylinder and a factor of 0.214 was used to transform bio-volume to DM (Smit *et* al. 1993).

- **C, N, P**

In each stream reach (C, I) samples were taken bi-monthly (4 before (B) and 5 after (A) the enrichment). Each sample had three replicates of the different trophic compartments: Coarse Particulate Organic Matter (CPOM), Suspended Matter (SM), biofilm, macroinvertebrates and fish. Samples were collected and processed according to the indications of Muñoz *et al.* (2009). Once obtained, the samples were refrigerated.

- CPOM

In the field, we separated three fractions of accumulated leaves from the stream bed.

- SM

A volume of 20 ml from the water column was filtered with Whatman GF / F filters that had previously been calcined (4 hours at 450°C).

- Biofilm

The biofilm was collected from 1 cm^2 ceramic tiles glued onto rock slabs that were kept at a depth of 10 to 20 cm in riffle zones for biofilm colonization (60 days). This colonization time ensured complete development of a biofilm similar to natural substrates (Donato *et al.* 2010). We cleaned the ceramic tiles in the lab with a toothbrush and added 10 ml of Milli-Q water. The sample was then sonicated in an ultrasonic bath for 5 minutes.

- Macroinvertebrates

For C,N and P analysis, invertebrate samples were taken from the Surber and corer samplers. The animals were sorted under a stereomicroscope and left for 12 hours in filtered river water under temperature-regulated conditions to clean their stomach content. Animals were pooled together for the analysis. On two occasions, one before (B. April 2008) and one after (A. January 2009) the enrichment, separated samples for some of the most common species (*Heterelmis* sp. (adult stage), *Tricorythodes* sp. and *Thraulodes* sp.) were analyzed.

- Fish

Samples were collected from the only two species found in the river *(Oncorhynchus mykiss* and *Trichomycterus bogotensis),* using an electro-shocker. We obtained a subsample of muscle tissue from each species of fish (three different animals for each sampling).

3.3.4. Sample Analyses

- **Preparation and analysis of C and N**

The samples obtained were placed in an oven heated to 60°C for three days and subsequently crushed with a mortar in order to obtain a homogeneous 0.1 mm. sample. The weight of the sample was obtained on a high-precision scale and it was then introduced into tin capsules into which a small amount (a spatula tip-full) of Vanadium Pentoxide was added as a catalyst. For liquid extracts from biofilm samples, we added 1 ml of the extract concentrate into the pre-weighed tin capsule, then dried and reweighed it and also added the catalyst. The capsules were stored on a dry rack until they were analyzed in a Thermo Elemental Analyzer 1108.

- **Analysis of P**

We obtained a known weight of the sample (2 to 20 mg), which was then placed in a test tube to which we added 20 ml of Milli-Q water and 2 ml of an oxidation reagent. Then sample was digested in the autoclave (90 minutes at 110 °C), and analyzed as a Phosphorus Reactive Soluble with the technique described in APHA-AWWA-WEF (2005).

3.3.5. Data Analysis

The effects on stream communities due to nutrient addition were determined by comparing the control and impact reaches both before and after the enrichment. This objective required the application of variance analysis using a BACI design: Before-After-Control-Impacted (Stewart-Oaten & Bence 2001). For said analysis, the physical, chemical and hydrological variable data were transformed to $\sqrt{x}$, density and biomass data were transformed into x^{-1}, and C, N and P percentage data were transformed into arcsin $\sqrt{x/100}$ to satisfy mathematical assumptions. The analysis was carried out using the SPSS 20 program.

3.4. Results

3.4.1. Physical, Chemical and Hydrological Variables

In general terms, physical and chemical variables presented similar conditions in the different reaches (C,I) and enrichment time (B,A) (Table 1). However, discharge variability during the period following enrichment was different from that of the previous period. During nutrient addition, maximum discharge values were lower and the minimum was higher with respect to the period before the addition. Moreover, just after the addition, discharge was clearly higher in impact reach (Figure 1). Significant differences were found in BACI analysis for nutrient concentrations, with Impact reach being higher after enrichment for NH_4^+ (n=26, F=4.685, P=0.042) and PO_4^{3-} (n=26. F=6.638, P=0.017) (Figure 2).

Table 1. Maximum (Max) and Minimum (Min) values of the physical, chemical and hydrological variables for the Control (C) and Impact (I) reaches Before (B) and After (A) nutrient enrichment.

	B				A			
	C		I		C		I	
Parameter	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**
Discharge (m^3s^{-1})	1.77	0.04	1.47	0.04	0.8	0.15	1.34	0.12
Temperature (T°)	15.9	12-4	17.51	11.9	14.57	13.03	15.47	13.19
Dissolved Oxygen (mg l^{-1} O_2)	8.01	7.08	8.11	6.84	7.89	7.54	7.87	7.22
Conductivity (µm cm^{-1})	1.75	39.67	1.97	4.2	133.67	52.67	152.67	51.67
pH	7.7	6.96	8.17	6.9	7.86	6.47	7.56	6.9
Ammonium (µg l^{-1} NH_4^+)	35.2	6.38	51.88	8.34	23.6	4.8	327.96	23.14
Phosphate (µg l^{-1} PO_4^{3-})	56.58	11.59	47.79	7.21	69.9	38.5	159.3	66.93
Nitrite (µg $l^{-1}NO_2^-$)	9.5	0.92	8.7	0.46	19.5	5.91	17.7	5.59
Nitrate (µg $l^{-1}NO_3^-$)	130.68	5.79	123.22	4.27	287.6	3.72	233.2	28

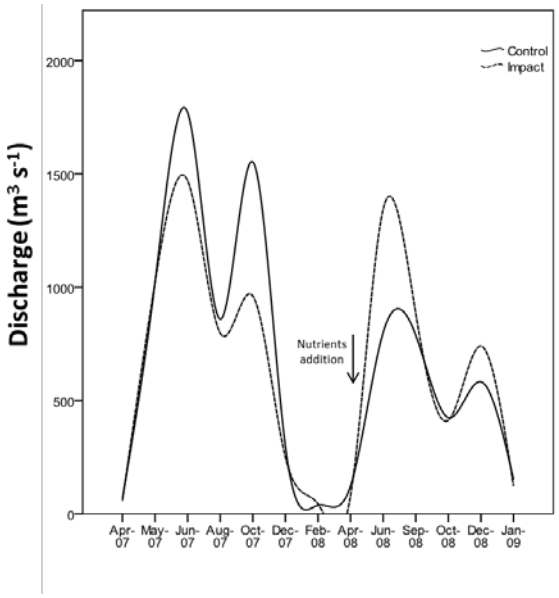

Figure 1. Discharge values of Tota stream for the Control (C) and Impact (I) reaches Before (B) and After (A) nutrient enrichment. Arrow indicates the time of nutrient addition.

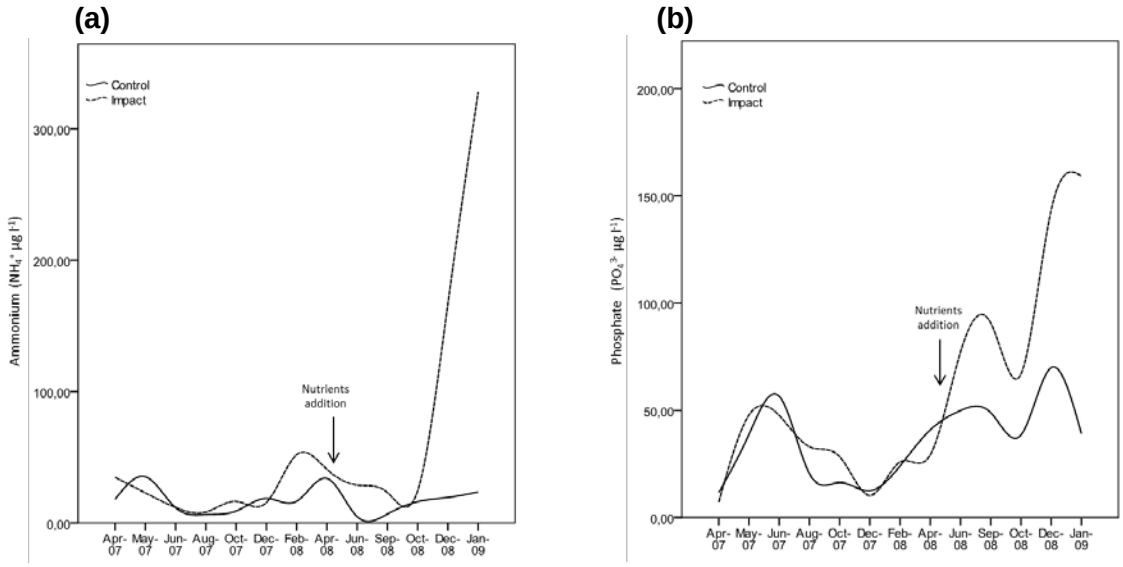

Figure 2. Ammonium **(a)** (NH_4^+) and Phosphate **(b)** (PO_4^{3-}) concentrations in the Control (C) and Impact (I) reaches during the experiment.

3.4.2. Biological analysis

- **Chlorophyll *a***

Mean values of chlorophyll *a* before enrichment were 2.76 μg m^{-2} (SD= 1.27) for C and a mean of 1.54 μg m^{-2} and (DS=0.35) for I. After the addition, the concentration in I (3.20 μg m^{-2} average and SD of 0.79) was 2 times the concentration in C (1.70 μg m^{-2} average and SD of 0.38). These differences were significant (BACI n=26, F=16.31, P=0.001) (Figure 3), showing the effect of nutrient enrichment on the periphyton biomass.

- **Abundance and Biomass of Macroinvertebrates**

Altogether, we have identified a total of 87 taxa (80 for rock and 44 for sand) in the two reaches (C,I) and periods (B,A). The most representative taxa are described in Tables 2 and 3 and they correspond to 94.95% and 97.66% of total density in rocks and sand respectively. In terms of biomass, these taxa correspond to 97.99% in rocks (Table 4) and 96.64% in sand (Table 5).

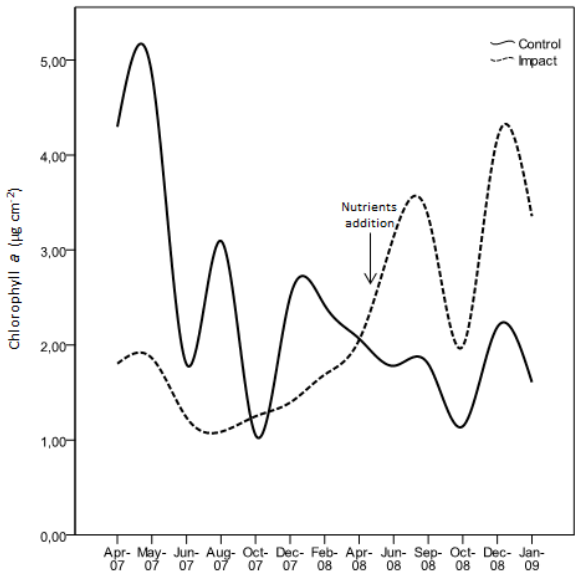

Figure 3. Chlorophyll *a* (μg cm^{-2}) concentration in the Control (C) and Impact (I) reaches during the experiment.

Figure 4 shows the total density and biomass values registered in this experiment. The highest density values were found in October 2008 for C and October 2007 for I, while in sand the highest values were observed in June 2007 for C and April 2007 for I. Biomass registered the highest values in rocks in June 2008 for C, in October 2008 and January 2009 for I, and for sand in June 2008 for C and May 2007 for I. In rocks at least, there was a tendency to increase total biomass, coinciding with periods of lower discharge values. Nevertheless, the BACI analysis showed no significant differences in density or biomass between reaches and periods in either of the habitats studied.

No differences were found when species were analyzed separately either. Nonetheless, despite the lack of significance, organisms like *Americobaetidius* sp. and *Camelobaetidius* sp. (Table 2) increased density and biomass in rock and sand habitats after enrichment. *Thraulodes* sp. demonstrated interesting behavior with an important density and biomass increase in rocks, while the values decreased in sand (Table 4). Unfortunately, the high variability between replicates limited the statistical significance of the results.

The dominant functional feeding group was that of collector-gatherers followed by collector-filterers (Figure 5). Shredder biomass in rocks was lower in the impact reach with respect to the control group. This group was not found in sand, and scrappers were also absent from sand in the impact reach. BACI analysis for functional feeding groups did not show significant differences.

• Stoichiometric relationships

The mean values of elements (C,N,P) and their molar ratios (C:N, C:P, N:P) are presented in Table 6 and in Figures 6, 7 and 8. The BACI analysis showed no significant differences between the stoichiometric values analyzed in the experiment. A slight increase in P content is observed in SM and CPOM in impact reach with respect to the control group. This enrichment was reflected in lower CP and NP values in these compartments (Figures 9 and 10). N is higher in biofilm after the enrichment in impact reach, and consequently lower CN ratio (Figure 6).

The t-test (contrast between both reaches after the enrichment) to analyze the stoichiometric ratios of selected species of collector-gatherers showed that *Heterelmis* sp.

did not present significant differences (Figure 10), whereas *Tricorythodes* sp. (t=2.969. P=0.041) presented significant differences for C:N ratio (Figure 11). *Thraulodes* sp. showed a significantly higher CN molar ratio (t=-10.974, P<0.001) (Figure 10).

Table 2. Values of mean and standard deviation (SD) of invertebrate density in rocks (ind m^{-2}) in the Control (C) and Impact (I) reaches during the experiment.

	B				A			
	Control		Impact		Control		Impact	
Taxa	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tubificidae spp	39	101.51	40	60.75	24	53.42	49	59.06
***Hyalella* sp.**	100	131.88	120	172.35	8	10.68	17	25.15
***Hydracarina* sp1**	23	30.64	72	209.08	7	17.72	19	31.89
***Americobaetis* sp.**	188	319.22	338	593.69	122	173.59	476	1036.72
***Camelobaetidius* sp.**	821	1384.41	2406	5599.82	396	449.56	2498	4755.46
***Leptohyphes* sp.**	86	84.45	102	308.56	90	126.98	156	190.75
***Paracloeodes* sp.**	145	394.11	287	676.27	37	83.53	122	188.82
***Thraulode*s sp.**	192	203.64	437	1266.77	311	248.82	1426	2574.92
***Tricorythodes* sp.**	699	1452.13	592	1029.98	158	213.34	491	826.94
***Culoptila* sp.**	175	313.87	258	441.39	72	134.95	114	159.65
***Hydroptila* sp1**	301	572.76	165	221.44	40	49.11	33	35.63
***Leucotrichia* sp.**	242	368.27	134	294.11	116	208.9	184	266.9
***Nectopsyche* sp.**	37	68.91	32	46.14	24	**53.75**	30	31.05
***Heterelmis* sp. (adult)**	36	62.27	40	91.49	8	10.68	10	13.59
***Heterelmis* sp. (larvae)**	67	52.11	64	63.04	30	32.44	36	48.46
Chironominae spp.	97	141.23	260	537.85	27	56.77	33	59.16
Orthocladinae spp.	304	315.75	246	249.38	173	194.61	267	273.79
***Simulium* sp.**	537	857.78	529	1375.55	903	1941.72	720	647.17
Tanypodinae spp.	42	42.24	43	46.41	16	24.07	20	31.2

Table 3. Values of mean and standard deviation (SD) of invertebrate density in sand (ind m^{-2}) in the Control (C) and Impact (I) reaches during the experiment.

	B				A			
	Control		Impact		Control		Impact	
Taxa	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Oligochaeta spp.	15	52.28	193	395.58	99	251.06	136	256.85
Tubificidae spp.	1898	2521.53	4583	8963.01	2469	3304.21	2889	5249.73
***Hyalella* sp.**	8	37.8	208	625.75	99	208.41	37	103.81
***Hydracarina* sp1**	147	313.59	46	226.8	543	1661.42	49	109.93
***Americobaetis* sp.**	123	330.66	62	140.99	86	118.5	210	327.29
***Camelobaetidius* sp.**	39	76.82	62	169.78	49	109.93	123	217.61
***Paracloeodes* sp.**	162	383.46	154	276.65	136	215.35	309	616.83
***Thraulode*s sp.**	62	169.78	46	112.57	235	492.27	123	249.1
***Tricorythodes* sp.**	548	1156.42	309	542.41	815	702.71	481	713.09
***Heterelmis* sp (larvae)**	185	244.21	324	568.14	160	349.02	74	136.44
Chironominae spp.	833	1514.29	8310	19095.56	469	537.01	1086	1749.6
Orthocladinae spp.	100	196.73	85	211.34	49	109.93	136	191.25
Podonominae spp.	154	481.24	571	1098.82	0	0	0	0
***Probezzia* sp1**	85	189	39	76.82	173	266.21	247	310.39
***Probezzia* sp2**	139	305.26	46	81.91	0	0	0	0
***Simulium* sp.**	39	94.25	131	492.91	62	166.61	99	169.53
Tanypodinae spp.	77	171.96	77	249.74	62	90.36	86	118.5
***Pisidium* sp.**	85	180.94	494	1874.74	99	183.41	185	474.71
***Physa* sp.**	8	37,8	154	647,65	0	0	0	0

Table 4. Values of mean and standard deviation (SD) of invertebrate biomass (mg m^{-2}) in rocks in the Control (C) and Impact (I) reaches during the experiment. Hydracarina spp1 was not measured.

	B				A			
	Control		Impact		Control		Impact	
Taxa	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tubificidae spp.	19	53.39	23	48.97	98	314.56	25	38.12
***Hyalella* sp.**	69	94.49	127	240.27	5	6.2	16	28.68
***Americobaetis* sp.**	232	356.45	463	861.49	108	167.61	2510	7790.88
***Camelobaetidius* sp.**	664	1010.84	1811	3736.08	401	411.28	2898	5409.3
***Leptohyphes* sp.**	46	63.91	67	185.93	34	47.14	135	267.12
***Paracloeodes* sp.**	166	338.01	356	878.19	43	98.95	146	275.65
***Thraulodes* sp.**	8843	19004.7	9876	17634.4	8650	7140.59	57658	132295.23
***Tricorythodes* sp.**	238	422.43	241	434.44	65	97.72	306	589.01
***Culoptila* sp.**	10	16.25	16	21.17	12	35.09	7	7.93
***Hydroptila* sp1**	35	69.4	14	20.02	3	4.03	3	4.87
***Leucotrichia* sp.**	29	44.46	15	32.81	13	21.91	20	25.73
***Nectopsyche* sp.**	18	47.77	17	27.79	5	8.57	4	3.95
***Heterelmis* sp. (adult)**	242	402.9	280	590.98	89	213.9	40	57.4
***Heterelmis* sp. (larvae)**	56	63.04	248	973.16	13	17.63	28	39.46
Chironominae spp.	11	19.16	26	57.26	3	8.43	1	2.74
Orthocladinae spp.	98	105.01	91	144.84	51	61.78	100	142.64
***Simulium* sp.**	765	1436.63	498	1487.47	562	1251.36	750	780.53
Tanypodinae spp.	15	16.24	21	25.84	10	14.71	10	18.14

Table 5. Values of mean and standard deviation (SD) of invertebrate biomass (mg m^{-2}) in sand in the Control (C) and Impact (I) reaches during the experiment. Hydracarina spp1 was not measured.

	B				A			
	Control		Impact		Control		Impact	
Taxa	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Oligochaeta spp.	67	232.35	3044	13769.83	2336	5895.82	684	1679.09
Tubificidae spp.	1478	2419.25	4106	8539.43	2095	2425.55	4190	7988.7
***Hyalella* sp.**	2	7.47	579	2411.49	59	118.5	72	189.13
***Americobaetis* sp.**	144	369.66	137	310.34	129	240.74	278	348.26
***Camelobaetidius* sp.**	43	90.15	85	233.21	88	190.55	196	367.72
***Paracloeodes* sp.**	160	389.89	259	532.62	177	321.58	276	631.1
***Thraulodes* sp.**	651	2025.47	838	3298.1	7513	16986.2	944	2050.74
***Tricorythodes* sp.**	264	520.23	316	723.52	571	609.21	147	225.48
***Heterelmis* sp. (larvae)**	162	244.14	402	829.98	147	284.46	52	103.45
Chironominae spp.	91	156.91	615	1875.28	31	31.89	60	81.36
Orthocladinae spp.	43	101.54	73	218.02	5	13.29	70	117.98
Podonominae spp.	62	147.23	345	951.1	0	0	0	0
***Probezzia* sp1**	27	68.44	14	34.93	63	91.16	111	145.26
***Probezzia* sp2**	57	151.22	26	47.83	0	0	0	0
***Simulium* sp.**	15	41.39	43	139.32	122	451.75	119	267.01
Tanypodinae spp.	37	112.34	73	281.4	35	53.89	35	70.57
***Pisidium* sp.**	82	257.56	286	1075.53	47	129.39	323	876.98
***Physa* sp.**	1	5.43	564	2468.93	0	0	0	0

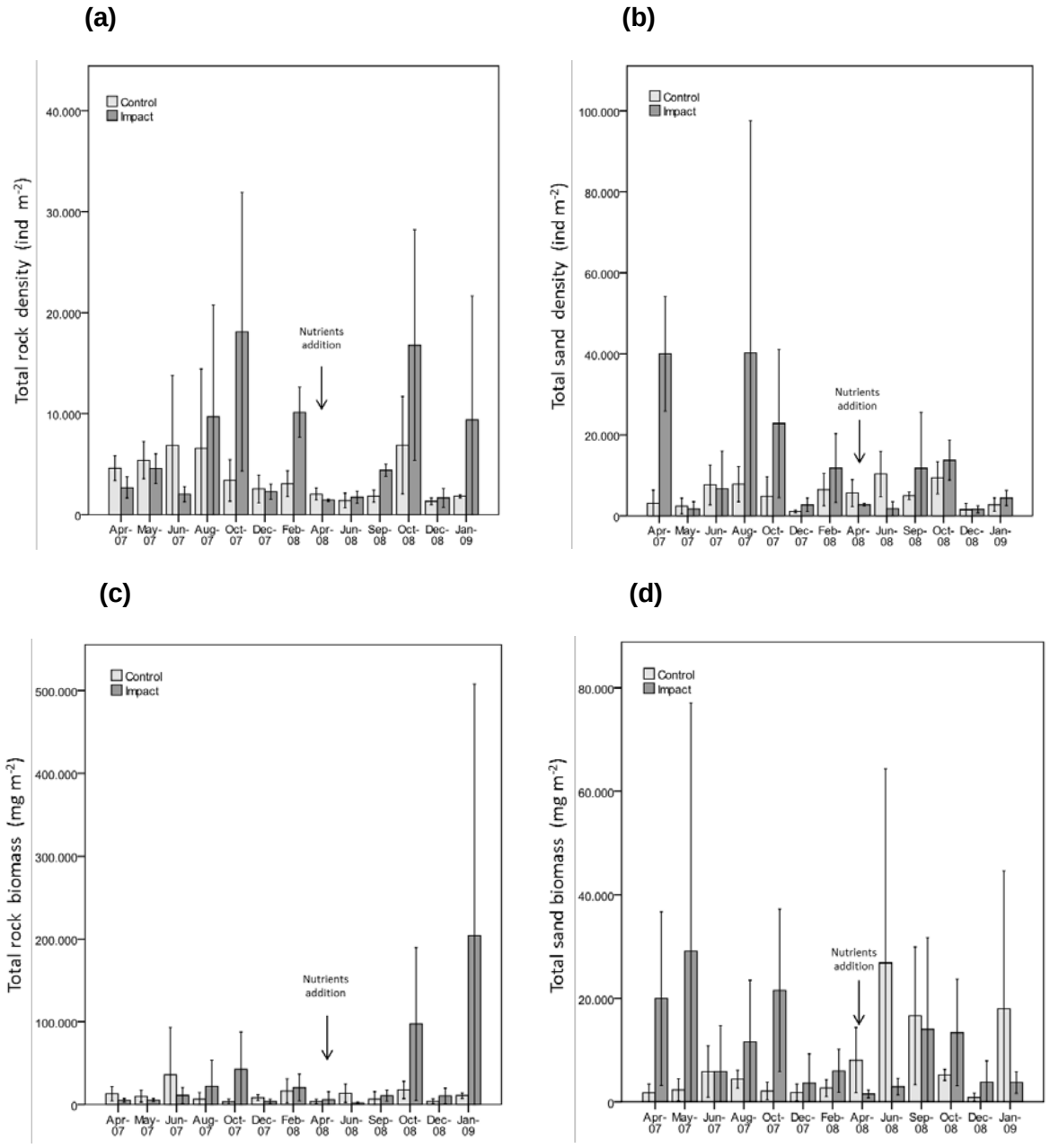

Figure 4. Total density (ind m^{-2}) and biomass (mg m^{-2}) in the Control (C) and Impact (I) reaches during the experiment: **(a)** density in rocks, **(b)** density in sand, **(c)** biomass in rocks, **(d)** biomass in sand. Arrow indicates the time of nutrient addition.

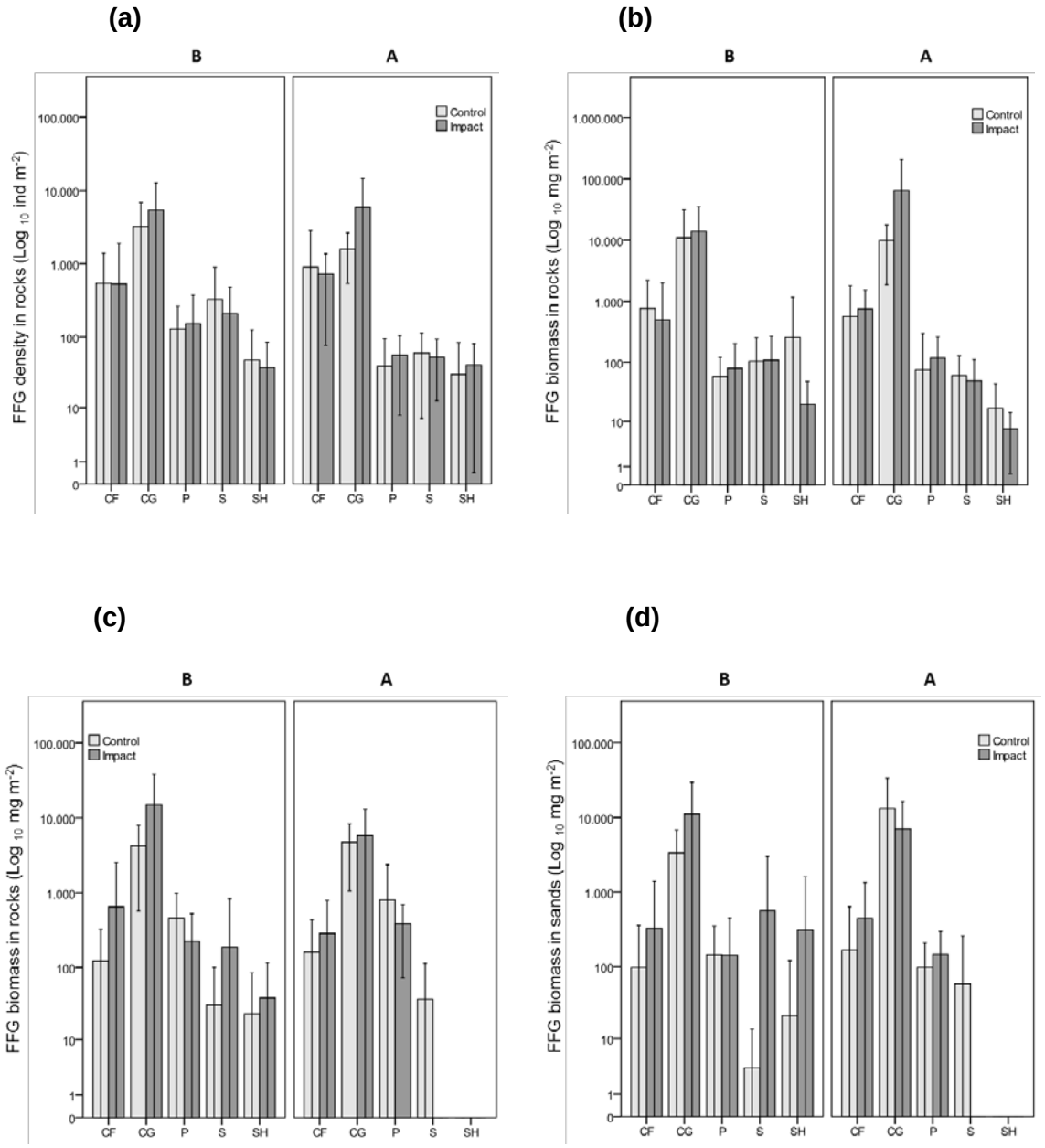

Figure 5. Density (ind m^{-2}) and biomass (mg m^{-2}) for functional feeding groups in the Control (C) and Impact (I) reaches during the experiment. Data was represented as a Log_{10} of the value: **(a)** density in rocks, **(b)** biomass in rock, **(c)** density in sand, **(d)** biomass in sand.

Table 6. Mean and standard deviation (SD) values of C, N and P percentages, and molar ratios (C:N, C:P, N:P)

		B				A			
		C		I		C		I	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
SM	%C	15.94	14.28	11.15	2.22	9.62	1.95	10.03	4.20
	%N	1.61	1.83	1.08	0.24	0.88	0.21	0.89	0.39
	%P	0.06	0.03	0.07	0.06	0.05	0.03	0.10	0.19
	C:N	13.04	1.83	12.17	2.10	12.90	1.43	13.25	1.00
	C:P	746.30	938.11	870.49	854.81	677.68	472.87	571.66	458.12
	N:P	67.06	100.24	65.59	53.69	53.64	38.68	43.73	37.47
CPOM	%C	46.41	3.71	45.70	4.67	43.69	5.59	45.67	4.94
	%N	1.77	0.51	2.32	0.73	1.64	0.54	1.82	0.64
	%P	0.05	0.03	0.03	0.02	0.04	0.02	0.06	0.03
	C:N	33.44	11.92	24.68	6.17	33.20	7.84	34.11	19.76
	C:P	3805.29	2070.06	4144.43	1840.53	3505.74	1567.63	2657.19	1375.10
	N:P	122.57	73.32	190.33	137.66	106.45	44.93	91.67	61.54
Biofilm	%C	6.32	4.83	3.62	2.09	9.10	7.45	9.69	5.49
	%N	0.55	0.47	0.25	0.09	0.90	0.81	1.09	0.75
	%P	0.05	0.02	0.04	0.02	0.07	0.04	0.08	0.05
	C:N	14.92	7.25	17.55	10.49	11.90	2.24	10.92	1.96
	C:P	338.25	179.44	270.59	124.23	334.01	228.89	317.08	91.02
	N:P	27.04	18.23	17.43	8.04	26.83	15.56	29.12	6.88
Macroinvertebrates	%C	44.77	5.22	45.14	4.81	47.53	3.11	46.17	3.66
	%N	7.81	1.08	7.56	1.45	8.14	1.47	7.46	1.19
	%P	0.21	0.09	0.24	0.13	0.19	0.16	0.17	0.13
	C:N	6.77	0.98	7.07	1.30	6.91	1.28	7.36	1.07
	C:P	675.16	384.68	676.90	473.84	1068.53	606.87	1028.29	494.58
	N:P	100.31	65.48	94.60	57.09	154.84	85.97	145.18	76.63
Fish	%C	46.32	4.90	42.14	14.49	48.94	1.51	49.45	2.00
Onchorynchus mykiss	%N	13.09	1.37	11.55	3.91	13.38	1.26	14.08	1.43
	%P	0.12	0.01	0.11	0.02	0.11	0.02	0.12	0.01
	C:N	4.14	0.27	4.25	0.28	4.30	0.45	4.14	0.49
	C:P	1045.89	192.24	1012.97	382.89	1220.67	313.02	1115.78	120.13
	N:P	253.21	44.84	238.14	89.46	286.53	84.64	272.50	38.63
Trichomycterus bogotensis	%C	51.04	3.92	50.59	6.91	54.33	2.11	53.70	1.70
	%N	10.69	1.25	10.38	1.26	13.13	2.92	11.25	1.20
	%P	0.11	0.06	0.30	0.45	0.19	0.06	0.10	0.01
	C:N	5.60	0.47	5.82	1.55	4.95	0.86	5.61	0.78
	C:P	1384.69	580.79	1125.81	648.05	801.09	316.27	1400.55	85.14
	N:P	246.46	96.03	191.75	110.91	160.03	44.36	253.10	50.16

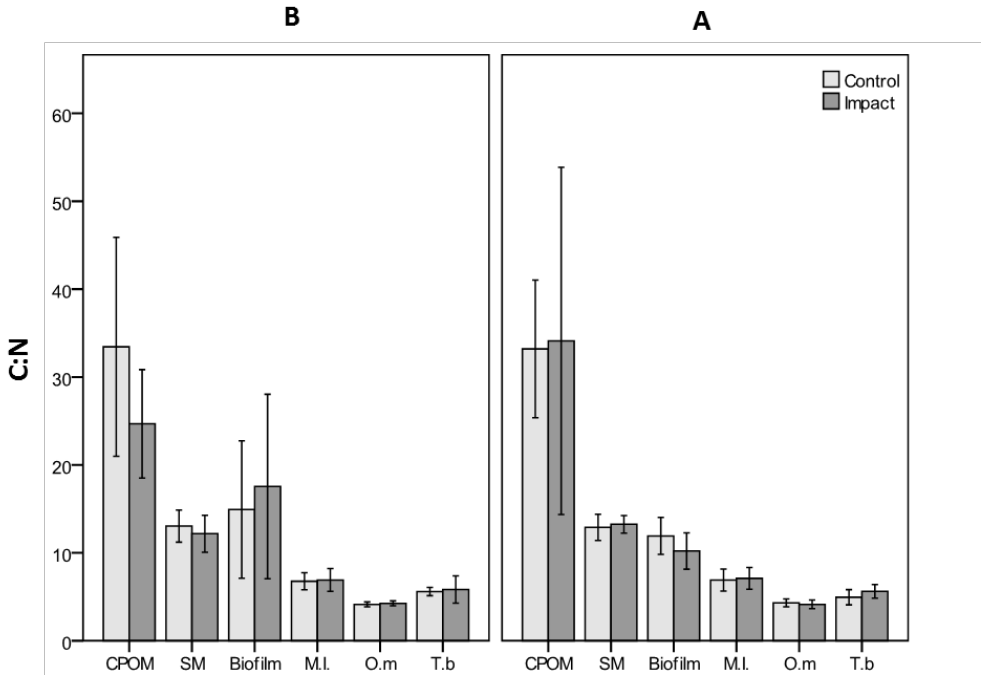

Figure 6. C:N molar ratio of different compartments analyzed in the Control (C) and Impact (I) reaches during the experiment. CPOM (Coarse Particulate Organic Matter), SM (Suspended Matter), M.I. (macroinvertebrates). O.m (*Onchorhynchus mykiss*), T.b (*Trichomycterus bogotensis*).

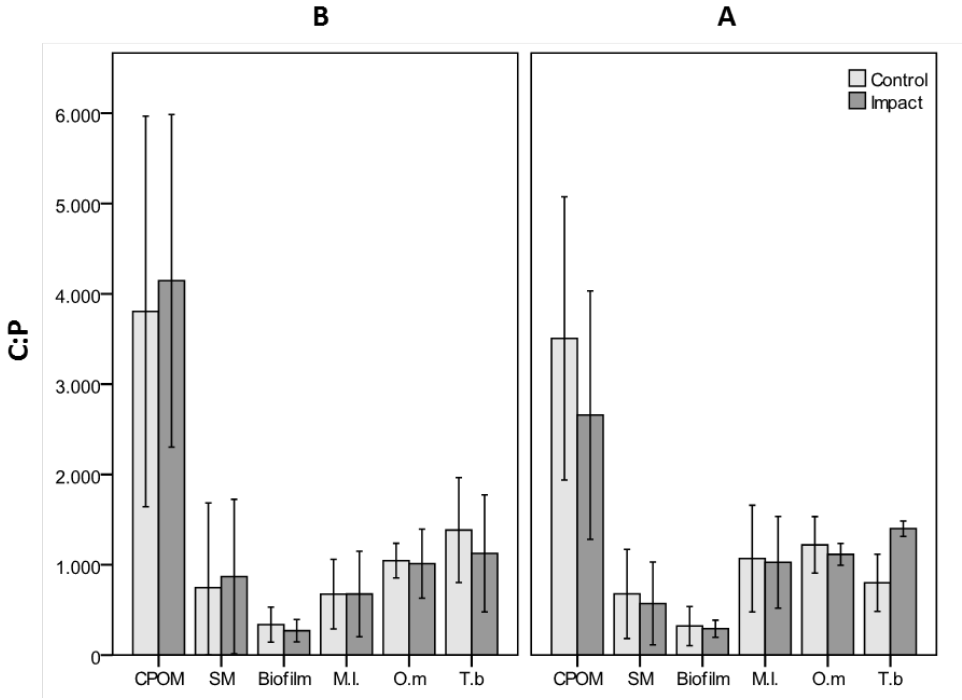

Figure 7. C:P molar ratio of different compartments analyzed in the Control (C) and Impact (I) reaches during the experiment. CPOM (Coarse Particulate Organic Matter), SM (Suspended Matter) M.I. (macroinvertebrates), O.m (*Onchorhynchus mykiss*), T.b (*Trichomycterus bogotensis*).

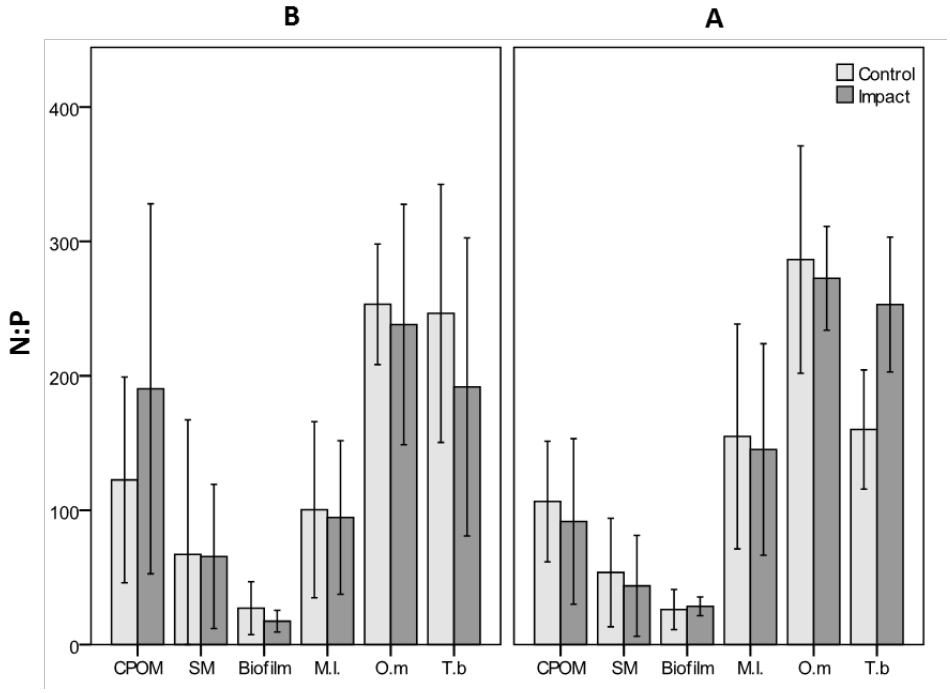

Figure 8. N:P molar ratio of different compartments analyzed in the Control (C) and Impact (I) reaches during the experiment. CPOM (Coarse Particulate Organic Matter), SM (Suspended Matter) M.I. (macroinvertebrates), O.m (*Onchorhynchus mykiss*), T.b (*Trichomycterus bogotensis*).

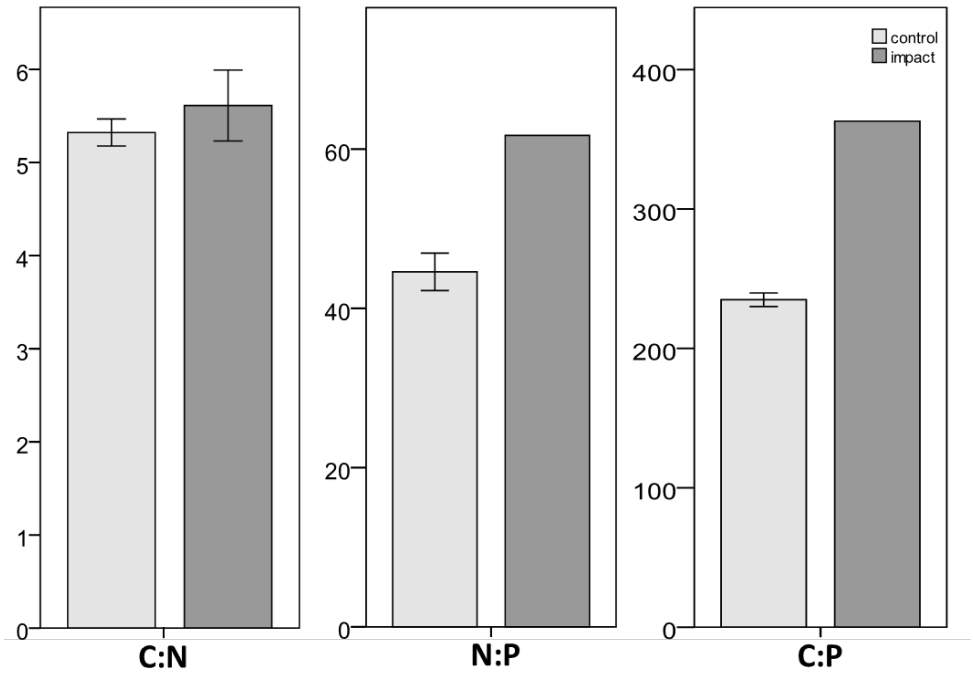

Figure 9. Stoichiometric ratios values of *Heterelmis* sp. during the enrichment period (A) in Control and Impact reaches.

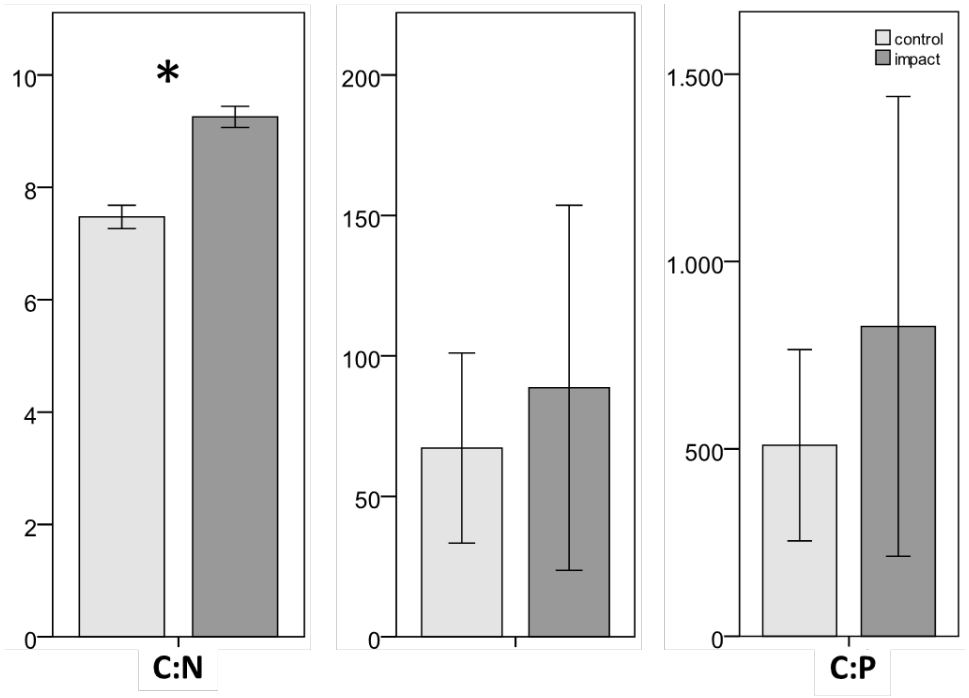

Figure 10. Stoichiometric ratios values of *Thraulodes* sp. during the enrichment period (A). *corresponds to significant differences between the Control and Impact reaches (P< 0.05) for t-student test.

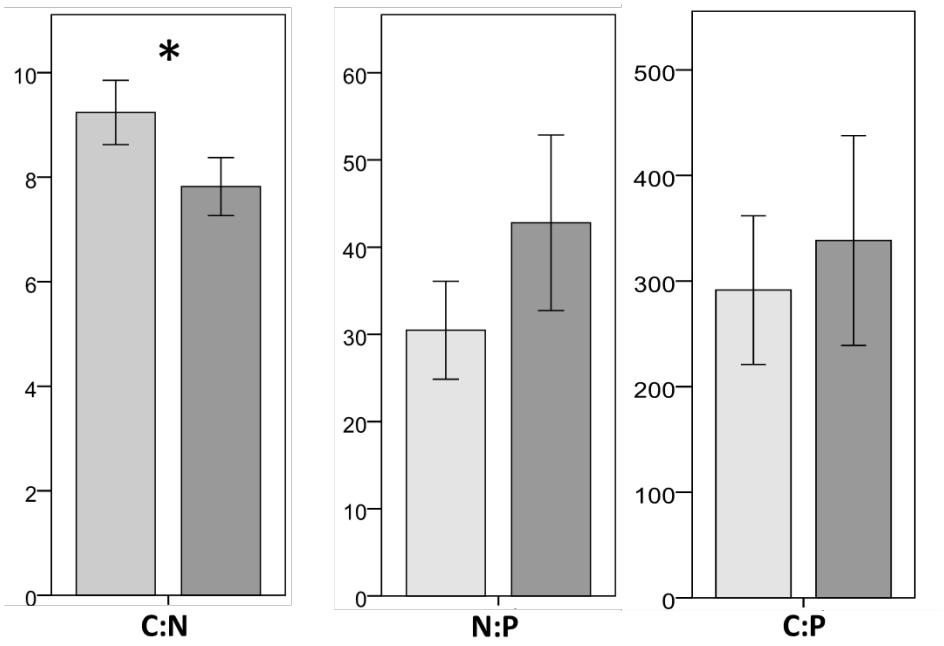

Figure 11. Stoichiometric ratio values of *Tricorythodes* sp. during the enrichment period (A). *corresponds to significant differences between the Control and Impact reaches (P< 0.05) for t-student test.

3.5. Discussion

As we hypothesized, the addition of nutrients had a positive impact on the increase of chlorophyll concentration. Tota stream is characterized by a low nitrogen concentration (Rivera & Donato 2008), and the addition favoured algae biomass measured as a chlorophyll *a* concentration. However, this effect was not significantly reflected either in consumer density or biomass. Only a slight increase was observed in invertebrate biomass in rocks.

It is clear that environmental variability and disturbance are regarded as key factors for community structure of invertebrates in streams (Stanford & Ward 1983, Townsend *et al.* 1 997). In our case hydrology plays an important role in the community structure and temporal patterns of Tota stream (Amaya 2008, Castro & Donato 2008b, and see chapter 1) as well as other high Andean mountain creeks (Rios-Touma *et al.* 2011 and Rios-Touma *et al*. 2012). Changes between high and low discharge periods could mask other effects, such as the effects of nutrient addition in this study. Low stability of sand with respect to hydrological changes could hamper the invertebrate response to nutrients, and hydrology could also limit consumer response in rocks, despite the increase of periphyton biomass. The lack of invertebrate community response to nutrient enrichment may have also been related to the time of addition – the longer the time, the greater the effects - and with species-specific characteristics such as life span or feeding type. For example, Sabater *et al.* (2011) described the effects for micro-crustaceans and grazers (*Ancylus*) and Cross *et al.* (2005a) did the same for chironomids. Both studies presented long-term additions (more than 3 years) and responses were related to the dominant food type.

Density values found in Tota were similar to those established by Ramírez & Pringle (1998) for lowland areas of Costa Rica, Flecker and Feifarek (1994) in Venezuela, and Rodríguez (2011) in the Sierra Nevada of Santa Marta (Colombia). However, biomass values of *Thraulodes* sp. were much higher than those reported by Ramírez and Pringle (1998) of 4.51 mg m^{-2} (DM). This species is one of the largest-sized macroinvertebrates in our stream.

Ephemeroptera dominates the density and biomass in the Tota stream, as was recorded in Ramírez and Pringle (2001), and Rodríguez-Barrios *et* al. (2007) for high mountain rivers of Colombia where the abundance of mayflies was significant in this kind of lotic

environments. In Tota these organisms represented more than 10% of the densities in rocks and over 80% of the biomass, while it had lower values in sand: 5% densities and 30% of the total biomass in the samples analyzed. The statistical analysis of density and biomass by Functional Feeding Groups (FFG) presented no significant differences, but a slight increment in density and biomass of collector-gatherers was evident, represented mainly by *Americobaetis* sp., *Camelobaetidus* sp. and *Tharulodes* sp., species that may take advantage of detritus and periphyton availability in impact reach after enrichment. Although they are collectors, they can consume algae from periphyton, as was described by Tomanova *et* al.(2006).

It is also noticeable that there was no accumulation of CPOM in the sandy parts of the stream after fertilization due to floods that occurred in this period. This lack explained the lack of shredders in said substrate.

In streams with allochthonous inputs, detrital carbon is often the major element available to consumers. In these systems the higher availability of N and P accelerates the carbon process by higher trophic levels. Growth and reproduction require a balanced diet between resources and individual demands for energy (Elser & Hessen 2005). Although not significantly, P content increased in suspended matter and in CPOM after the enrichment in our experiment, which probably coincided with increases in microbial production in detritus. In the same way, biofilm increased its N percentage. However, the stoichiometric molar ratios showed no significant changes when BACI analysis was used in relation to the experiment. However, these results only partially support our previous hypothesis since we expected clearer changes in detritus and biofilm quality.

Comparing with Cross *et al.,* (2003) we found that the C:P and C:N molar ratios for SM and CPOM were slightly lower in Tota but in the same order of magnitude, however N:P was higher. Biofilm in Tota showed significantly (one order of magnitude) lower C:P and N:P. Tota had higher phosphorous concentration than the headwater streams (around 7 μg/L) studied by the former authors at the basal conditions.

The C:N, C:P and N:P values found in Tota for stream collectors (analyzed separately) were in the range than those reported by Cross (2003), Bowman *et al.,* (2005), or Small & Pringle (2010). However, the C:P and N:P ratios of fishes were higher respect to those described by McIntyre and Flecker (2010), showing a limitation of P for top predators in Tota. This low relative P content in fish may mean evolutionary adaptation to low quality of

food resources, or diversification of diet (e.g. algae) to complement nutrient supply (Sterner & Elser 2002).

Macroinvertebrate stoichiometric analysis was performed using a pool of all the species mixed together in the sample that prevented us from clearly seeing the effect of enrichment on this compartment. Cross *et* al. (2003) has already described differences in stoichiometric ratios with taxa in streams. Different orders and genera presented different ratio values. When we conducted a t-test of the CNP ratios separately for some collector-gatherer species, only *Tricorythodes* sp. showed a significant decrease in C:N ratio in impact reach after nutrient enrichment. The slight response of resources to nutrient addition would explain the lack of response in consumers. The trends observed could indicate that a longer period of enrichment had been necessary to find more clearly response in the community.

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Chapter 4

Food Web of a Tropical High Mountain Stream: Effects of Nutrient Addition

4. Food Web of a Tropical High Mountain Stream: Effects of Nutrient Addition

4.1. Abstract

Using a nutrient enrichment experiment in an Andean mountain stream, we used stable isotope ratios (δ^{15}N and δ^{13}C) to analyze different trophic compartments: 1) basal level: CPOM and biofilm; 2) primary consumers – macroinvertebrates: collector-gatherers (*Heterelmis* sp, *Thraulodes* sp and *Trichorythodes* sp), and collector-filterers (*Simulium* sp); 3) predators – fish (*Oncorhynchus mykiss* and *Trichomycterus bogotensis).* The average fractionation of nitrogen among the primary consumers with respect to CPOM was 4.7‰ and 1.7‰ with respect to biofilm. Predators incremented their δ^{15}N signal by 5.9% with respect to primary consumers*.* A depletion of δ^{15}N was observed in Impact with respect to control reach after fertilization in different compartments (biofilm, *Heterelmis* sp*.*, *Simulium* sp. and *Tricorythodes* sp*.)*, while depletion was not significant for top predators. In most cases, the δ^{13}C signal of biofilm overlapped with that of primary consumers, but a clear enrichment was observed with respect to CPOM. The macroinvertebrates referred to were selected to analyze their gut content and the results showed us that fine detritus is the most abundant food in invertebrates, and only *Heterelmis* sp. showed significant differences in fine detritus and vegetal matter between control and impact reaches after the nutrient addition.

Keywords: food webs, nutrient enrichment, isotope ratios, trophic compartments, gut content.

4.2. Introduction

Food webs are complex trophic connections among interacting organisms in ecosystems (Elser & Hessen 2005), and their structure influences population dynamics, community structure and ecosystem function (Polis *et al.* 1997). A knowledge of the food web in freshwater systems is essential to integrate the dynamics of organic matter with organism interactions.

The maximum food-chain length is an important food-web property and has been correlated with resource availability, ecosystem size, environmental stability and colonization history (Doi 2011). Some of these correlations may result from environmental effects on predator–prey mass ratios (Jennings 2005). As well as providing information about connections in the system, food-web diagrams can be used to study community structure, ecosystem processes, and forms of bioaccumulation of contaminants (Post 2002).

In streams, the structure of food webs is affected by numerous factors, such as biogeography, stream order, disturbance, temperature, resource type and, obviously, by anthropogenic activities (Hershey *et al.* 2007 and references therein). Resources in streams are usually represented by detritus and primary producers that are always associated with fungi, bacteria and micro and meiofauna. In this way, invertebrates can be both primary and secondary consumers. Predators are usually omnivorous with a mixed diet of prey, detritus and algae. These characteristics produce confusing results that make it difficult to determine the resources assimilated by animals.

Stable isotope analysis has proved to be a useful tool in reconstructing diets, elucidating patterns of resource allocation, characterizing trophic relationships and constructing food webs (Boecklen *et al.* 2011), thus providing a measurement of trophic position that integrates the assimilation of energy or mass flow through all the different trophic pathways leading to an organism (Post 2002). Carbon and Nitrogen stable isotopes are frequently used to study energy sources and food web structure in ecosystems (Bergfur *et al*. 2009), as well as to show which processes or components are more sensitive to perturbation (Peterson & Fry 1987).

Furthermore, $\delta^{15}N$ isotopes are useful for differentiating trophic levels and food-web dynamics (Berfurg *et al.* 2009) because $\delta^{15}N$ isotopic fractioning increases with each trophic level (Finlay 2001) and a consumer is typically enriched by 3-4‰ relative to its diet, although different studies have found lower values for $\delta^{15}N$ fractionation (Jardine *et al.* 2012). In contrast, the ratio of carbon isotope ($\delta^{13}C$) changes little (0.3-0.5 ‰ average) as carbon moves through food webs (Peterson & Fry 1987), and is an effective diet tracer because there is little fractionation associated with trophic transfer of organic carbon in food webs (Finlay *et al*. 2002). Analysis of $\delta^{13}C$ signature has an advantage over gut-

content analysis because it measures the amount of carbon assimilated from each food source as opposed to that ingested (March & Pringle 2003). However, as we have mentioned before, due to high overlapping in the diets of stream organisms, gut contents provide basic and complementary information about food sources.

The aim of this study is to identify the links between sources and consumers in an Andean mountain stream. We analyze the stable isotope ratios (δ^{15}N and δ^{13}C) of different trophic compartments present in two reaches, one of which was subjected to a nutrient enrichment experiment. We hypothesized that nutrient enrichment increases nitrogen and phosphorous composition of the basal compartments, mainly in algae, which improves their quality for consumers. This better quality favors basal resource consumption for all trophic levels in the enriched reach, and is expected to lower δ^{15}N values.

4.3. Methods

4.3.1. Experimental Design. Please, refer to previous chapter.

4.3.2. Hydrological, Physical and Chemical Variables. Please, refer to previous chapter.

4.3.3. Biological Sampling

Two samplings were carried out in each stream reach (C, I), one before (B, April 2008) and one after (A, January 2009) the enrichment. Three replicates of the different trophic compartments (Coarse Particulate Organic Matter (CPOM), biofilm, macroinvertebrates and fish) were taken on each occasion. Samples were collected and processed according to the indications of Muñoz *et al*. (2009) and Hershey *et al*. (2007). The samples were refrigerated between sampling and lab processing.

- **Coarse Particulated Organic Matter**

We collected three fractions of accumulated leaves from the stream bed in the field.

- **Biofilm**

Ceramic tiles (1 cm^2) glued onto rock slabs were located at both reaches and kept at a depth of 10 to 20 cm in riffle zones for biofilm colonization (60 days). This colonization time ensured a complete development of biofilm similar to natural substrates (Donato-Rondón *et al*. 2010). In the lab, we cleaned the ceramic tiles with a toothbrush and added 10 ml of Milli-Q water. Each sample was sonicated in an ultrasonic bath for 5 minutes.

- **Macroinvertebrates**

Samples were taken in rock substrata using a Surber sampler with a 900 cm^2 surface area and 500 µm net mesh size. In the lab we then sorted the animals and left them in filtered river water under temperature-regulated conditions for 12 hours to clean their stomach content. For isotope analysis, we selected the most abundant species: *Heterelmis* sp. (adult stage), *Simulium* sp., *Tricorythodes* sp. and *Thraulodes* sp.

- **Fish**

Fish were sampled using an electro-shocker. Individuals from the only two species found in the river *(Oncorhynchus mykiss* and *Trichomycterus bogotensis)* were collected, from which we obtained a subsample of muscle tissue.

4.3.4. Sample Analyses

- **Preparation and Analysis of $\delta^{13}C$ and $\delta^{15}N$**

The extract of samples were dried at 60°C for three days and were then crushed with a mortar to obtain a homogeneous sample of 0.1 mm particle size. For liquid extract samples of biofilm, we added 1 ml of the extract concentrate onto the pre-weighed tin capsule, and then dried and reweighed it. All the samples were subsequently packed into

tin capsules and stored in dried conditions. Samples were analyzed in a Thermo Elemental Analizer 1108 associated to a mass spectrometer. Standards specified by the International Atomic Energy Agency (IAEA) were used to calibrate the isotopic signal: sucrose, polyethylene and graphite for carbon; ammonium sulphate and potassium nitrate for nitrogen. The standard test was run repeatedly to ensure linearity. The results were compared with the isotopic composition of atmospheric nitrogen for nitrogen, and Pee Dee belemnite carbonate rock (PDB) for carbon as reference.

- **Gut-Content Analysis**

The same species that we chose for $\delta^{13}C$ and $\delta^{15}N$ analysis were selected in order to analyze their gut content. Fish gut contents were not analyzed. After organism collection, the animals were deposited in plastic bags before being frozen. Five individuals of each species were analyzed. The individuals were placed in vials containing rose Bengal for 24 hours. Afterwards, the digestive tract of each individual was extracted and the anterior part was removed under a stereomicroscope. This material was then placed on a slide to be studied under a microscope (400x). Ten visual fields were selected at random and photographed. The photos were quantified (for percentage of different categories, see above) using the Coral Point (CpCe 3.4) program. Five categories of food sources were identified: coarse detritus (CD), fine detritus (FD), diatom algae (DA), filamentous algae (FA) and vegetal matter (VM) (Vegetal Matter).

4.3.5. Data Analysis

Isotopic signals were represented in a bi-plot figure using the SIGMAPLOT 10 program. Differences in isotopic signals and in category percentages in gut content were tested with a t-test to find differences between reaches and time separately.

4.4. Results

4.4.1. Physical, Chemical and Hydrological Variables. Please, refer to previous chapter.

4.4.2. Stable Isotopes

The $\delta^{15}N$ signal clearly established three trophic levels in the reaches studied throughout the experiment: 1) basal level with CPOM and biofilm; 2) primary consumers (macroinvertebrates: collector-gatherers (*Heterelmis* sp., *Thraulodes* sp. and *Trichorythodes* sp.) and collector-filterers (*Simulium* sp.); 3) predators (fish, *Onchorhynchus mykiss* and *Trichomyterus bogotensis*, Figures 1 and 2).

Table 1 shows the mean values and Standard Deviations (SD) of the isotopic signals in the different compartments. The $\delta^{15}N$ of consumers was enriched, compared to primary sources - mainly CPOM - in different proportions, depending on the feeding habits of each taxa. The CPOM presented similar isotopic values in all phases of the experiment and constituted an indicator of the base of the food chain. However, the biofilm showed a higher $\delta^{15}N$ signal (between 3.5 and 4‰) than CPOM, except for the treatment reach after the nutrient addition where the $\delta^{15}N$ was 0.10‰. Biofilm is usually composed of heterotrophic elements (bacteria, fungi, microorganisms) in addition to autotrophs (algae) that can enrich the nitrogen isotopic ratio. The average fractionation of nitrogen of the primary consumers with respect to CPOM is 4.7‰ (minimum 3.8 and maximum 5.5‰) and 1.7‰ with respect to biofilm (ranging between 0.3 and 4.0‰). Values for *Tricorythodes* sp. $\delta^{15}N$ in impact reach after enrichment have not been taken into account in this range due to their unusual low values). Predators increased their $\delta^{15}N$ signal by 5.9‰ (from 4.3 to 10.17‰) with respect to primary consumers (Figure 1, Table 1).

In most cases, the $\delta^{13}C$ signal of biofilm overlapped with that of primary consumers, but a clear enrichment is observed with respect to CPOM. Following the $\delta^{13}C$ and the gut contents results (see below), one would predict that almost all the invertebrates analyzed

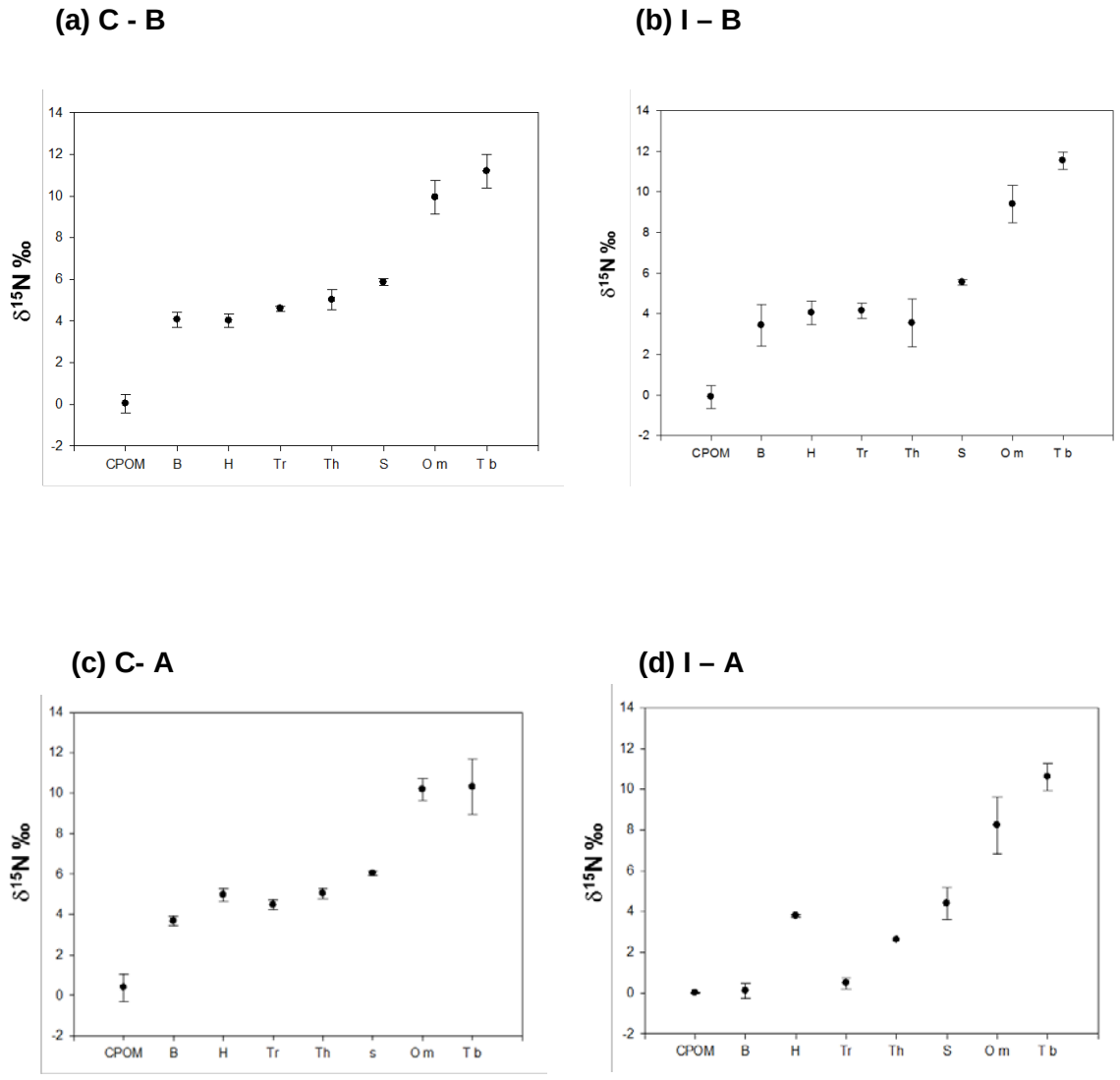


Figure 1. δ^{15}N values of CPOM, biofilm (B), macroinvertebrates: *Heterelmis* sp. (H), *Tricorythodes* sp. (Tr), *Thraulodes* sp. (Th), and *Simulium* sp. (S); and fish: *Oncorhynchus mykiss* (O m), *Trichomycterus bogotensis* (T b) in Control (C), Impact (I) reaches, Before (B) and After (A) the nutrient addition. *Thaulodes* sp. in IA is a single sample.

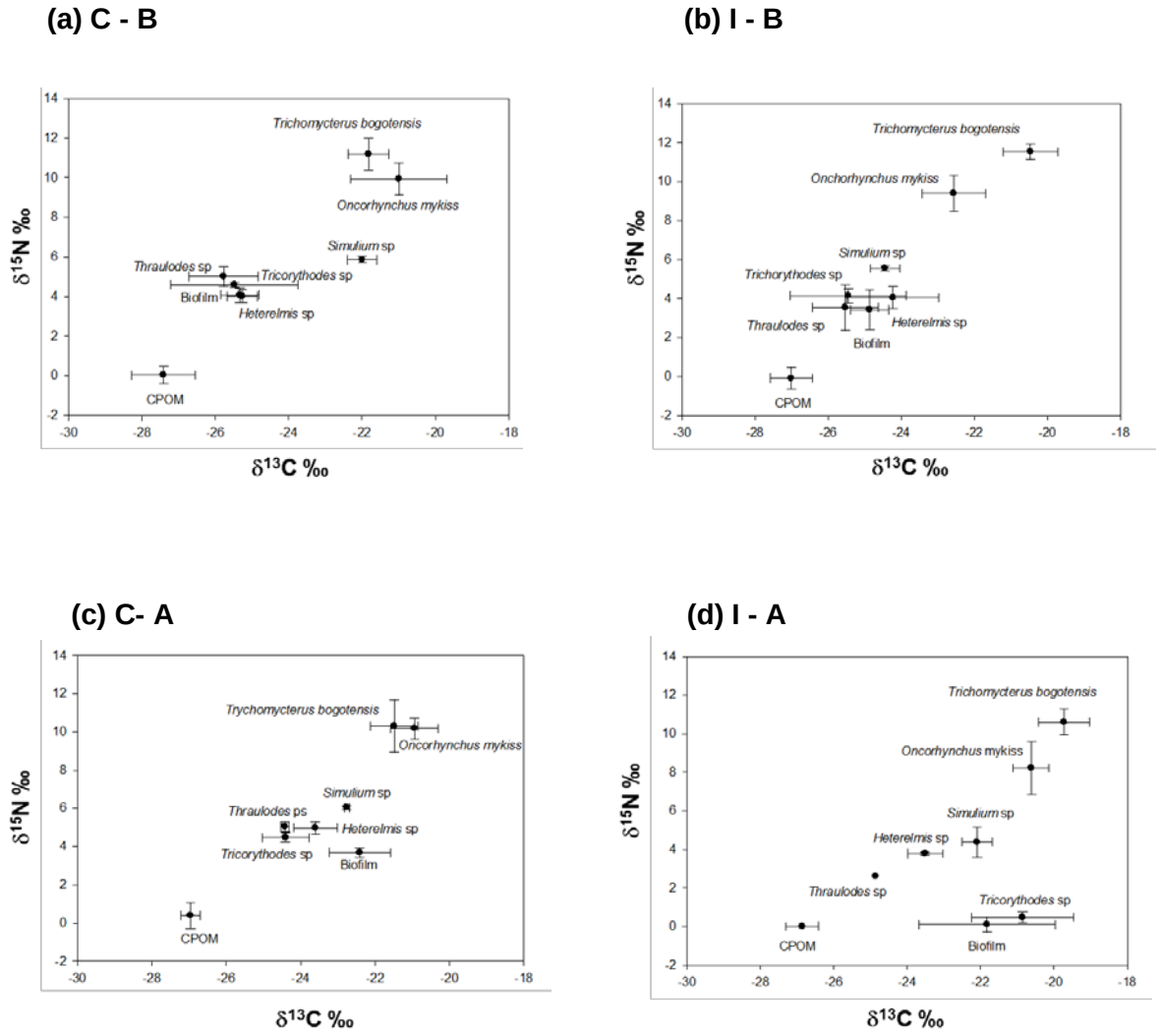


Figure 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}C$) signatures of basal resources (biofilm, CPOM), macroinvertebrates (*Heterelmis* sp., *Thraulodes* sp., *Tricorythodes* sp. and *Simulium* sp.) and fishes (*Oncorhynchus mykiss*, *Trichomycterus bogotensis*) in Control (C), Impact (I) reaches, Before (B) and After (A) the nutrient addition. Points are means ±1 SD. *Thaulodes* sp. in IA is a single sample.

were actually feeding on CPOM and biofilm (Figure 2). An increase in $\delta^{13}C$ values of biofilm was observed in both reaches after the enrichment (n=3, t=-5.259, p= 0.006 in control reach and t= -2.944, p=0.05 in I reach), indicating an enrichment of ^{13}C with respect to ^{12}C, probably related to environmental changes (e.g. flow).

Oncorhychus mykiss and *Trichomycterus bogotensis* feed on macroinvertebrates in both reaches, and in I reach *Oncorhychus mykiss* became a potential prey of *Trichomycterus bogotensis* ($\delta^{15}N$ fractionation: 2.14‰ and 2.32‰ Before and After respectively), showing that there are differences in habitat conditions for the development of live stages of the silurid fish between the two reaches.

A depletion in $\delta^{15}N$ was observed in I with respect to C reach after fertilization in different compartments: biofilm (t= 13.453, p= 0.001), *Heterelmis* sp. (t= 5.572, p= 0.01) *Simulium* sp. (t= 4.019, p= 0.02) and *Tricorythodes* sp. (t= 17.42, p< 0.001). Biofilm reflected the use of inorganic N from fertilizer ($\delta^{15}N$= 0,1 ± 0 ‰) and invertebrate signals in its consumption. This depletion was not significant for top predators.

Table 1. $\delta^{13}C$ and $\delta^{15}N$ values of the different compartments for the Control (C) and Impact (I) reaches, Before (B) and After (A) nutrient enrichment.

		B				A			
		C		I		C		I	
Compartment	**Isotope**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
CPOM	$\delta^{13}C$	-27.41	0.87	-27.01	0.57	-26.96	0.26	-26.85	0.44
	$\delta^{15}N$	0.04	0.44	-0.09	0.57	0.39	0.69	0.01	0.01
Biofilm	$\delta^{13}C$	-25.33	0.52	-24.86	0.53	-22.41	0.82	-21.82	1.85
	$\delta^{15}N$	4.07	0.36	3.43	1.01	3.68	0.24	0.10	0.37
***Heterelmis* sp**	$\delta^{13}C$	-25.27	0.41	-24.22	1.26	-23.60	0.59	-23.51	0.47
	$\delta^{15}N$	4.02	0.32	4.04	0.57	4.98	0.31	3.79	0.07
***Thraulodes* sp**	$\delta^{13}C$	-25.78	0.94	-25.53	0.90	-24.42	0.11	-24.86	
	$\delta^{15}N$	5.02	0.49	3.54	1.18	5.04	0.27	2.61	
***Tricorythodes* sp**	$\delta^{13}C$	-25.48	1.74	-25.45	1.59	-24.40	0.63	-20.86	1.38
	$\delta^{15}N$	4.60	0.12	4.14	0.37	4.47	0.24	0.48	0.29
***Simulium* sp**	$\delta^{13}C$	-21.99	0.41	-24.44	0.39	-22.76	0.07	-22.09	0.40
	$\delta^{15}N$	5.86	0.17	5.55	0.13	6.04	0.10	4.38	0.79
Oncorhynchus mykiss	$\delta^{13}C$	-20.99	1.31	-22.56	0.87	-20.94	0.64	-20.62	0.49
	$\delta^{15}N$	9.93	0.82	9.39	0.93	10.19	0.54	8.22	1.38
Trichomycterus bogotensis	$\delta^{13}C$	-21.82	0.55	-20.46	0.76	-21.48	0.64	-19.72	0.70
	$\delta^{15}N$	11.19	0.81	11.53	0.41	10.31	1.36	10.61	0.67

4.4.3. Gut Contents

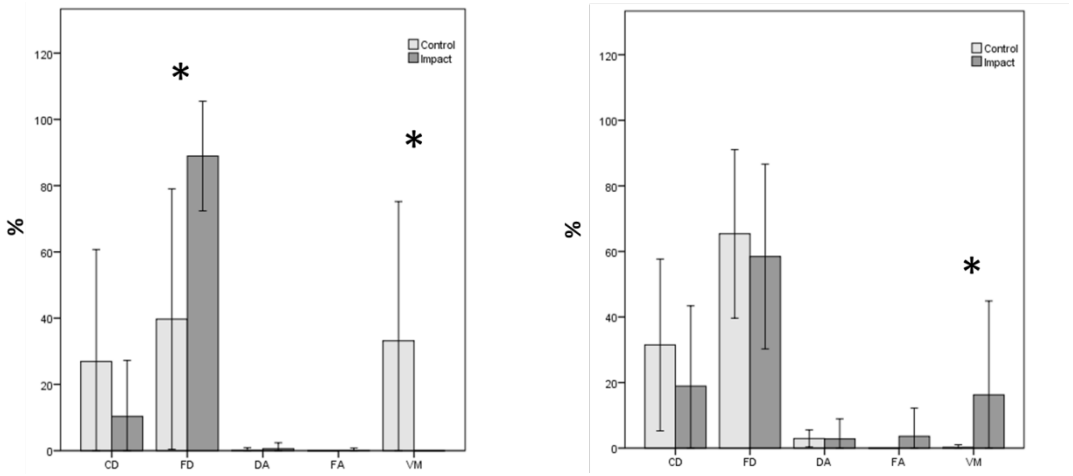
Fine detritus (FD) is the most abundant food in the four invertebrate gut contents analyzed. Proportions of algae (diatom and filamentous algae) were always 10% lower than the gut content. *Heterelmis* sp. showed significant differences in FD (n= 5 t=-5.159 , P < 0,01) and VM (n= 5, t=3.533, P=0.001) between control and impact reaches after the nutrient addition. *Simulium* sp. in VM (n=5, t=-2.496, P=0.017), *Thraulodes* sp. in CD (n= 5, t=6.760, P<0.001) and FD (n= 40, t=7.027, P<0.001). *Tricorythodes* sp. did not show significant differences in any of the food categories (Figure 3).

4.5. Discussion

In spite of progress in the study of isotopes in food webs, there is little information available regarding tropical high mountain systems. In our study, the Tota stream food web shows about three to four trophic levels according to the spatial work scale of the study (Post *et al.* 2000). This high mountain creek has great hydrological dynamics, nutrient limitations (Rivera & Donato 2008) and low productivity (Abuhatab 2011) that could be important factors for limiting connectivity between species (Schmid-Araya *et al.,* 2002, Jardine *et al.,* 2012).

Comparisons between Tota stream and other tropical systems show that the $\delta^{13}C$ values obtained for biofilm (-25.33 to -21.82)) and CPOM (-27.41 to -26.85) are similar, although slightly higher than those reported for leaves in other small tropical streams (March & Pringle, 2003; Dudgeon *et al.,* 2010). In addition, our values correspond to those given by Peterson and Fry (1987) for C_3 plants. Several factors can affect algal fractionation of C (Finlay *et al.* 2002), given a broad range of values. Fine detritus is derived from both algal and detrital components and has intermediate $\delta^{13}C$ values (Hershey *et al.* 2007). It is an important source for consumer diet, as is observed in our study of gut contents, but, unfortunately, we did not analyze this compartment for stable isotopes that would complement the total basal resources and their consumer relationships in this stream.

(a) *Heterelmis* sp. **(b) *Simulium* sp.**



(c) *Thraulodes* sp. **(d) *Tricorythodes* sp.**

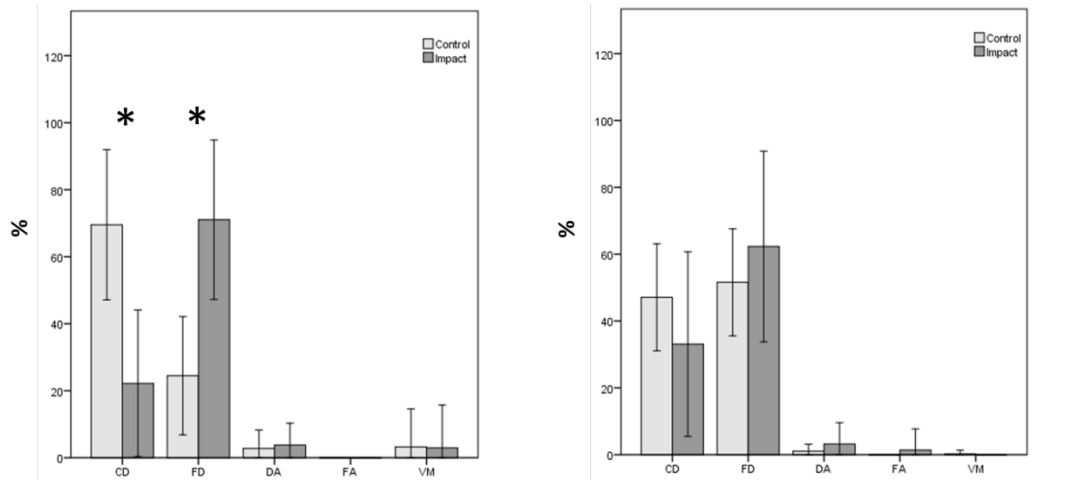


Figure 3. Gut content percentage found in macroinvertebrates by using isotope analysis during the enrichment period. * corresponds to significant differences between the Control and Impact reaches ($P < 0.05$) for the t-student test. CD (coarse detritus), FD (fine detritus), DA (diatom algae), FA (filamentous algae), VM (vegetal matter).

It is very difficult to obtain clean samples of periphyton from the field since the algae cells grow into the biofilm matrix together with bacteria, microfauna and detritus accumulation, thus obtaining higher values of $\delta^{15}N$ than other basal resources. This makes the calculation of Nitrogen fractionation for primary consumers with respect to resources more difficult. Values in our system are near those predicted in the literature (an average of 3.4‰, Post 2002) although with high variability (ranging between 0.3 and 4.0‰) when biofilm is used in calculations. Fish nitrogen fractionation values were an average of 5.9‰ and clear $\delta^{13}C$ enrichment was observed with respect to the invertebrates, showing their feeding dependence.

We found a strong relationship between collector-gatherers and biofilm in the two reaches (C,I) and periods (B,A), indicating strong reliance on algal carbon in this feeding group. *Tricorythodes* sp was the collector-gatherer that had the closest connection with the biofilm observed with the common $\delta^{15}N$ depletion. A previous study (Donato-Rondon *et al.* 2010) has already shown that this species is strongly associated with periphyton resources, while Tomanova *et al.* (2006) has indicated that most invertebrate collectors in tropical rivers are not food specialized and their dietary changes are related to the availability of resources.

Finlay *et al*. (2002) argues that trout isotopic ratios vary seasonally, depending on food resource availability. In our case, *Onchorhynchus mykiss* ratios were clearly related to primary consumers in the two samplings and reaches. In the case of our other top predator, *Trichomycterus bogotensis,* it is important to establish that no previous studies have focused on its biology and dietary habits, but only on taxonomical aspects. However, records of diets presented by Habit *et al.* (2005) and Roman-Valencia (2001) for *T. areolatus* and *T. caliensis,* respectively, define an insectivore behavior. In our study it is evident that diet is composed by insects and in the impact reach also by *Oncorynchus mykiss.* In the same way, Chará *et al.,* (2006) determinate that *Trichomycterus* spp. is insectivore and could be a piscivore organism. For this particular species it is important to establish biological aspects to define its habitat requirements because a difference in the type of predatory behaviour was evident between study reaches (C,I) with similar prey availability. We observed that in Impact reach this species was more abundant in the deeper pools located within said reach. This habitat condition may change predatory behavior.

An increase in $\delta^{13}C$ values of biofilm was observed in both reaches after the nutrient addition, indicating an enrichment of ^{13}C with respect to ^{12}C, probably related to environmental changes. This increase could be a product of discharge temporality (Before samples were taken at the beginning of the high discharge period and the After ones were taken in the low discharge period). In this period a thicker periphyton, active photosynthesis, combined with diffusion-limited movement from the water to cells, is likely to cause the depletion of inorganic carbon within the periphyton matrix and higher values of $\delta^{13}C$ (Hill & Middleton 2006). On the contrary, Hladyz *et al.* (2011) raises the point that maximum enrichment in cobble biofilm $\delta^{13}C$ signature occurred following periods of high discharge, while maximum depletion occurred during the low discharge period. These findings highlight the fact that trophic links between basal resources and primary consumers can be altered profoundly and that changes in hydrology will alter food chains and energy fluxes to the higher trophic levels (Perkins *et al.* 2010).

As we have hypothesized, nutrient addition, partly in nitrogen form, depletes the $\delta^{15}N$ signature of biofilm and of most of the primary consumers, reflecting their dependence on this source, although no evidence of higher consumption was observed in gut contents. Conversely, detritus entering from riparian forest will not be depleted. Moreover, the proportion of fine detritus in gut contents significantly increased in *Heterelmis* sp. and *Thraulodes* sp. after the addition. Both results may indicate a higher consumption of fine detritus and biofilm in the impacted reach, even though no clear significant differences were found in their quality (for stoichiometric ratios, see previous chapter) due to fertilization. However, this depletion is not reflected in fish. Their high mobility along the river (Jardine *et al*., 2012) and long life cycle would lead to different patterns for these top predators.

The natural distribution of isotopic abundance in Tota stream gives us information about the links between a resource or prey and predator in the two reaches and periods studied, and the nitrogen addition works as a tracer approach confirming those links. Complementary to this, the gut-contents data help to decide which link is the most correct and to discern dietary changes due to nutrient addition.

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Chapter 5

Effects of Eutrophication on the Interaction Between Algae and Grazers in an Andean Stream

5. Effects of Eutrophication on the Interaction Between Algae and Grazers in an Andean Stream

5.1. Abstract

Nutrient excess is a common disturbance that affects biological interactions in river ecosystems. The response of nutrient supply on primary producers and *Tricorythodes* sp., a common mayfly grazer, was determined in experimental chambers set in a tropical, high Andean stream. Chambers in a experimentally fertilized reach developed higher amount of both benthic and detached chlorophyll than chambers in an upstream control reach. Fertilization produced a slight increase in grazer biomass, and reduced algal biomass compared to grazer-free chambers. These results show that nutrient excess spread bottom-up effects through the food web, and that relevant top-down effects could also be detected. Eutrophication may produce relevant changes in the food web of tropical high-mountain streams.

Keywords: Algal biomass – Colombian Andes – *Tricorythodes* sp. – Chambers – Nutrient enrichment.

5.2. Introduction

During the 70‘s, it was assumed that biomass and productivity of a certain trophic level were defined by the predation and the grazing at higher levels (effects "Top-down"; Steinman 1996). The predominant control over the distribution and abundance of algae in rivers is given from higher trophic levels (herbivores) to the lower trophic levels (biofilm, benthic algae); however, this control depends upon time, place and the environmental conditions (Rosemond *et al*. 1993, Allan & Castillo 2007). Many experiments established that grazing can significantly affect the structure and dynamics of primary producers’ communities (Lamberti & Resh 1983, Lamberti & Moore 1984, Hart 1987, Hill & Knight 1987, Liess & Hillebrand 2004, Peters *et al.* 2007). Manipulative experiments carried out on the interaction and effects between the herbivorous insects and periphyton in rivers

have been limited mostly to caddisflies (Lamberti & Resh 1983, McAuliffe 1984). Hill & Knight (1987) carried out a study on the interaction among the mayfly *Ameletus validus* and the periphyton in a small north California stream. All these studies reported a reduction and substantial alteration in periphyton growth, primary production and community structure. Other experiments reported the exclusion of herbivorous insects from periphyton (Lamberti & Moore 1984, Murphy 1984, Hart 1985, Hill & Knight 1987),

Nutrient excess is one of the most common disturbances affecting river ecosystems, through “bottom-up” effects to the whole community structure *(*Biggs & Smith 2002). In recent years, both "bottom-up" or "top-down" control of the communities of algae, have been considered important for the structure of trophic webs (McQueen *et* al. 1986) and the effects of both nutrients and herbivores (Hillebrand & Kahlert 2001, Hillebrand *et al.* 2002).

The Andean tropical fluvial ecosystems lodge an enormous biological diversity. The marked seasonal variation in the temperature characteristic of the temperate systems is substituted by altitudinal variation, and the differences in the rainy and drought periods influence the hydrological dynamics (Zapata & Donato 2005). The incident solar radiation is intense despite of local conditions. In the Andes, the radiation is 50% higher that in the sea level for a regime of equivalent atmospheric humidity (Lewis *et al.* 1995).

Original forests are fragmented and less than 30% of their original extension remains (Armenteras *et* al*.* 2003). The particular effect nutrient enrichment in the biodiversity and structure of communities in tropical rivers, and in particular in the Andean region, is largely unknown. These fluvial systems face increasing human population densities and rising eutrophication that add to the seasonal flow variations (Donato & Galvis 2008).

This article aims to solve the consequences of nutrient enrichment on the relationships between algae and nutrients, as well as the potential implications for the trophic net. The use of controlled experiments has been the main tool to analyze the effect of the nutrient enrichment in the ecological interactions and the biodiversity. This type of experiments may be essential in understanding the response of rivers and streams of tropical fluvial systems. In particular the main objective of the article was to determine the relationships between nutrient supply, algal biomass and its link with *Tricorythodes* nymphs grazing in

controlled experiments. Results may be indicative of effects of fertilization in Andean streams.

5.3. Methods

5.3.1. Experimental Design

After Chapter 3 and 4 nutrient addition experiment, another experiment was carried out in 30 m of the impact reach. The first 15 m of the reach were kept in natural conditions (control reach), while in the other 15 m (fertilized or impact reach) nutrients were added by using a 500 l tank. Two commercial grain fertilizers were diluted in the tank (Nitron 26 (26-0-0) and Abocol (NPK) (10-30-10)) in order to rise at least two times the average basal (natural) phosphates (1.93 µg l^{-1}) and ammonium concentrations (16.12 µg l^{-1}) in the stream.

Twenty-four chambers were placed in the fertilized reach and in the control reach. These chambers were made of transparent acrylic material, and were 7 cm length, 7 cm height and 7 cm width. Each chamber had three circular openings of 3.5 cm of diameter covered with a 0.5 mm mesh, to allow continuous flow of water through them (Figure 1). Fertilization started 15 days before introducing the animals in the chambers. In that period chambers were placed in the stream to allow periphytic colonization.

In each treatment reach, we randomly choose 12 chambers and introduced 10 *Tricorythodes* sp. nymphs into each chamber. The *Tricorythodes* sp. were collected in the study area. Their average body length was 3.0 mm and the cephalic capsule was 0.5 long by 0.5 mm wide. The herbivores were placed in the compartments at the beginning of the experiment, when the periphyton colonization period was finished.

The sampling was carried out after 3, 10, 17 and 28 days of introducing *Tricorythodes* sp. On each sampling day, six chambers were randomly selected for sampling from both the

fertilized and control reaches. Of the six chambers taken in each treatment reach, three were chambers without grazers and the remaining three contained grazers.

Tricorythodes sp. nymphs were taken out of the chambers every sampling day and immediately measured. Extracted chambers were wrapped up in aluminium paper and moved to the laboratory for the estimation of benthic chlorophyll *a* and detached periphyton chlorophyll *a*. Chlorophyll was measured from the detached periphyton and from that suspended in the chamber water by using the APHA, AWWA & WEF (2005).

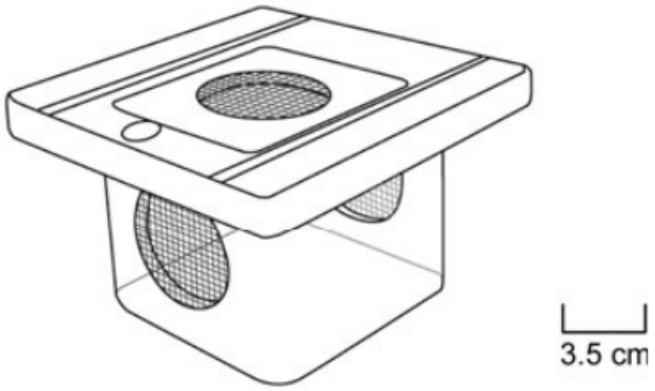


Figure 1. Chamber used as the artificial substrate in the experiment

5.3.2. Hydrological, Physical and Chemical Variables

We use the same methods mentioned in Chapter 2. However, for this experimental design discharge was measured daily and the light values were measured with a Model LI-COR LI-250[a] luxometer.

5.3.3. Data Analysis

Before beginning the experiment, 83 *Tricorythodes* nymphs were collected. In each animal sampled were measured the total body length, length and width of the cephalic capsule.

They were oven dried (48 h at 70 °C) and weighed with a precision of 0.01 µg. The results were fitted to the equation by Burgherr & Meyer (1997),

$$DM = a * L^{b}$$

where *a* and *b* are the regression constants, DM dry mass (mg) and L total body length (mm). *Tricorythodes* length was related to biomass with the equation:

$$\ln DM = -7.45 + 4.06 \ln(L)$$
$$(n=83, r^2=0.66, p<0.0001)$$

This equation was used to define the initial biomass of the 240 nymphs of *Tricorythodes* sp. introduced in 24 chambers (12 in the control and 12 in the fertilized reach) and to compare it with the final biomass of the individuals extracted throughout the experiment.

A multivariate analysis of variance (MANOVA) was carried out to detect significant differences between treatments by using the SPSS 15.0 for Windows.

5.4. Results

5.4.1. Environmental conditions

The reaches showed average values of 0,05 $m^3 s^{-1}$ discharge, 7.05 pH, 166.69 µS cm^{-1} conductivity, 7.31 mg L^{-1} dissolved oxygen, 16.87 ºC temperature, 102.48% oxygen saturation and 907.16 µmol $m^{-2} s^{-1}$ environmental light during the experimental period. The control and fertilized reaches showed significant differences in physical conditions among days (Table 1, Figure 2), but not between them (Table 2). Only dissolved oxygen was significantly different between days at the two reaches. The dissolved oxygen showed a similar behaviour between reaches during the first days of the experiment (fertilized reach with an average of 7.4 mg l^{-1} and control reach with an average of 7.2 mg l^{-1}) until day 25. The oxygen values were slightly higher from day 25 onwards in the fertilized (7.0 – 7.9 mg l^{-1}) than in the control reach (6.9 – 7.6 mg l^{-1}).

The fertilization obviously produced a significant increase in nutrient availability (Figure 3, Table 2). Concentration of ammonia (NH_4^+) rose from 16.12 µg l^{-1} to 265.11 µg l^{-1}, and phosphate (PO_4^{3-}) from 1.93 µg l^{-1} to 66.37 µg l^{-1}.

Table 1. Maximum and minimum values among physical, chemical and hydrological variables between control and fertilized reach.

	Control reach		Fertilized reach	
	Min	**Max**	**Min**	**Max**
Discharge ($m^3 s^{-1}$)	0.01	0.32	0.01	0.37
Temperature (°C)	14.5	20.2	14.4	20.3
pH	5.45	7.84	5.25	7.68
Conductivity (µS cm^{-1})	89.00	224.00	89.00	244.00
Dissolved Oxygen (mg L^{-1})	6.65	7.56	6.77	7.98
Light (µmol $m^{-2} s^{-1}$)	235.40	1410.63	258.07	1412.53
NH_4^+ (µg L^{-1})	16.12	37.68	14.22	265.11
PO_4^{3-}(µg L^{-1})	1.93	42.7	1.37	66.37

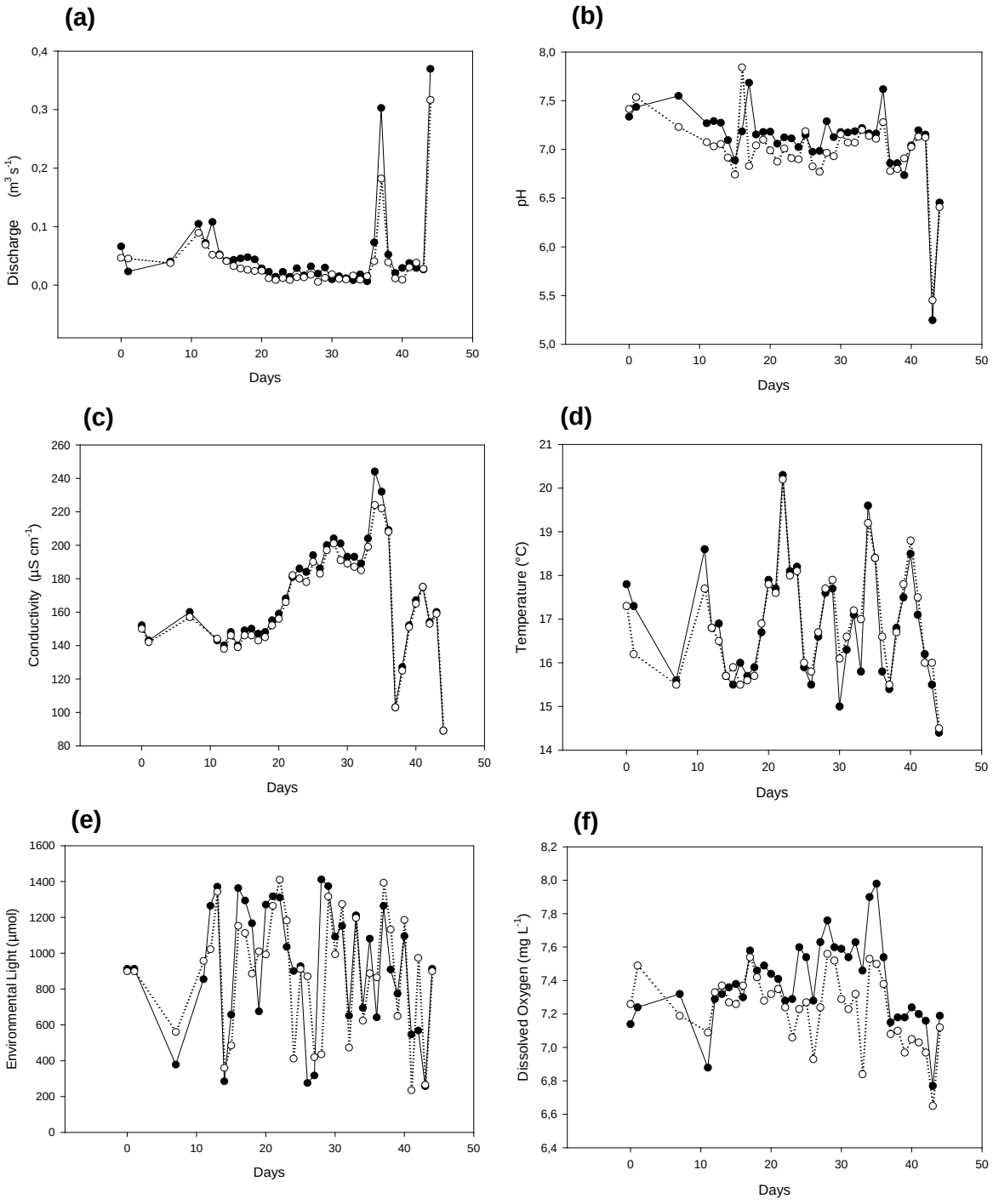


Figure 2. (a) Discharge, **(b)** pH, **(c)** Conductivity, **(d)** Temperature, **(e)** Environmental light and **(f)** Dissolved oxygen values in the sampled days, control (○) and fertilized (●) reach.

Table 2. Using ANOVA analysis, significance values (p<0,05) among the physical, chemical, and hydrological variables between treatments (control- fertilized) and the sampled days.

	Reaches (Fertilized– Control)		Sample Days	
	F	p	F	p
Discharge ($m^3\ s^{-1}$)	0.736	0.394	23.291	<0.0001
pH	1.409	0.239	8.924	<0.0001
Conductivity ($\mu S\ cm^{-1}$)	0.210	0.648	156.615	<0.0001
Dissolved Oxygen ($mg\ L^{-1}$)	7.326	0.008	2.915	0.001
Temperature (°C)	0.022	0.883	31.005	<0.0001
Light ($\mu mol\ m^{-2}\ s^{-1}$)	0.160	0.691	5.784	<0.0001
PO_4^{3-}($\mu g\ L^{-1}$)	21.496	<0.0001	1.389	0.162
NH_4^+ ($\mu g\ L^{-1}$)	50.277	<0.0001	0.454	0.990

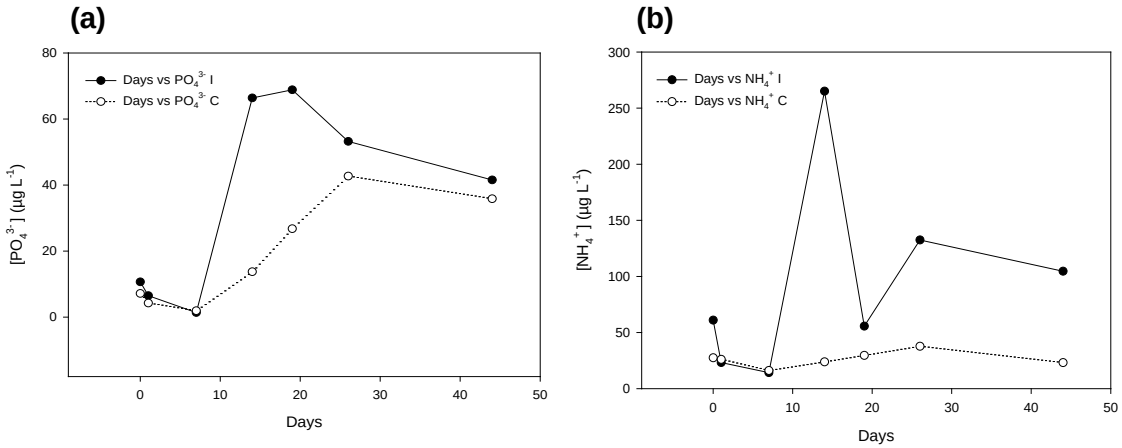


Figure 3. Increment of (a) Phosphate (PO_4^{3-}) and (b) Ammonium (NH_4^+) concentrations in the fertilized (●) and control reach (○) during the study days.

5.4.2. Biota

Significantly higher concentrations of benthic (n=24, F=242.54, p<0.0001) and detached periphyton chlorophyll *a* (n=24, F=52.52, p<0,0001) were observed because of fertilization, (Figure 4).

In chambers with *Tricorythodes* sp. in the control reach the benthic chlorophyll *a* showed values of 0.18 µg cm^{-2} (day 19) – 0,39 µg cm^{-2} (day 33). Chlorophyll concentrations ranged between 1.00 µg cm^{-2} (day 19) to 2,47 µg cm^{-2} (day 44) in the fertilized reach. The detached periphytic chlorophyll *a* showed values of 0.07 µg cm^{-2} (day 19) to 0.58 µg cm^{-2} (day 33) in chambers of the control reach and from 0.15 µg cm^{-2} (day 19) to 2.79 µg cm^{-2} (day 33) in the fertilized reach.

In the chambers without *Tricorythodes* sp. the benthic chlorophyll *a* ranged between 0.12 µg cm^{-2} (day 19) to 0.45 µg cm^{-2} (day 44) in the control reach and 0.56 µg cm^{-2} (day 26) to 2.27 µg cm^{-2} (day 44) in the fertilized reach. The detached periphytic chlorophyll *a* concentration corresponded to 0.10 µg cm^{-2} (day 19) to 0.87 µg cm^{-2} (day 44) in the control reach and 0.22 µg cm^{-2} (day 19) to 4.78 µg cm^{-2} (day 44) in the fertilized reach.

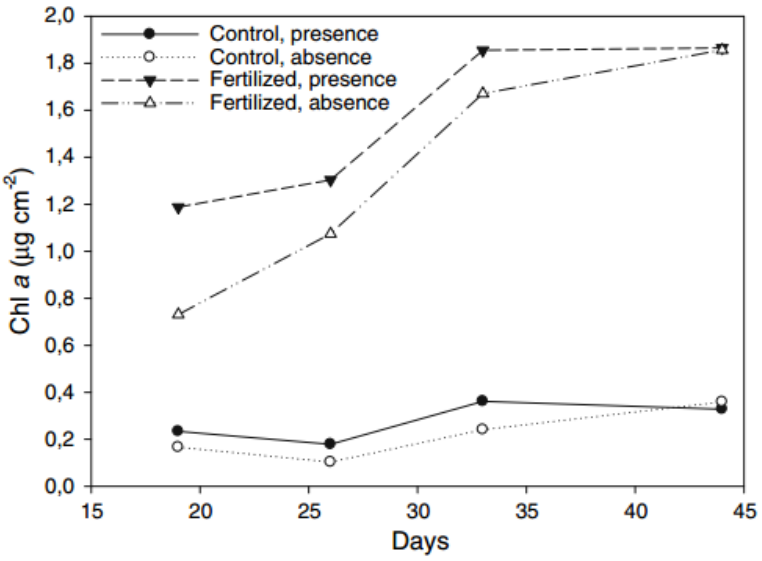
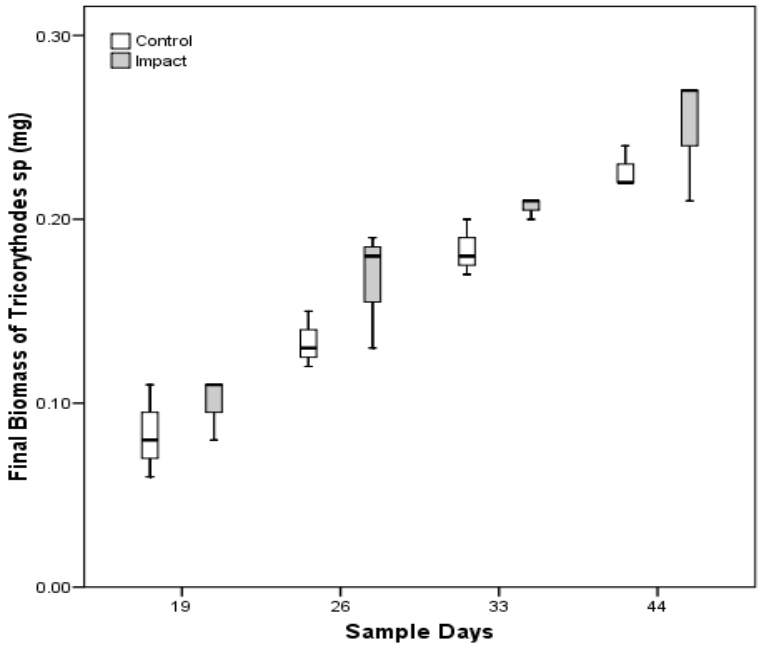


Figure 4. Concentration of benthic chlorophyll *a* in fertilized and control boxed in presence and absence of grazers.

Average mayfly biomass was initially 0.06 mg at the control reach and 0.07 mg at the fertilized one. The final biomass did not present any significance difference (n= 12, F=0.979, p= 0.333) between the two reaches. However, when the mean light value measured in each box was used as covariable differences became statistically significant (n= 6, F= 12.255, p= 0.003). Differences were also significant when biomasses between sampling days were compared (n= 6, F= 42.752, p<0.0001) because of the rise of the nymphs final biomass in the fertilized reach in relation to the control reach (Figure 5). Benthic chlorophyll *a* did not show significant differences regarding the biomass of mayfly nymphs (n= 12, F= 4.985, p= 0.037).

Overall, the detached periphyton chlorophyll *a* was significantly higher in the chambers with mayfly nymphs (n= 24 F=22.300, p<0.0001). We observed that detached periphyton chlorophyll *a* concentrations were higher in the impact than in control treatments. In the presence of mayfly nymphs the chlorophyll *a* concentrations were lower than in the treatments without the herbivore (Figure 6).



Figures 5. Biomass of *Tricorythodes sp.* in fertilized and control reaches.

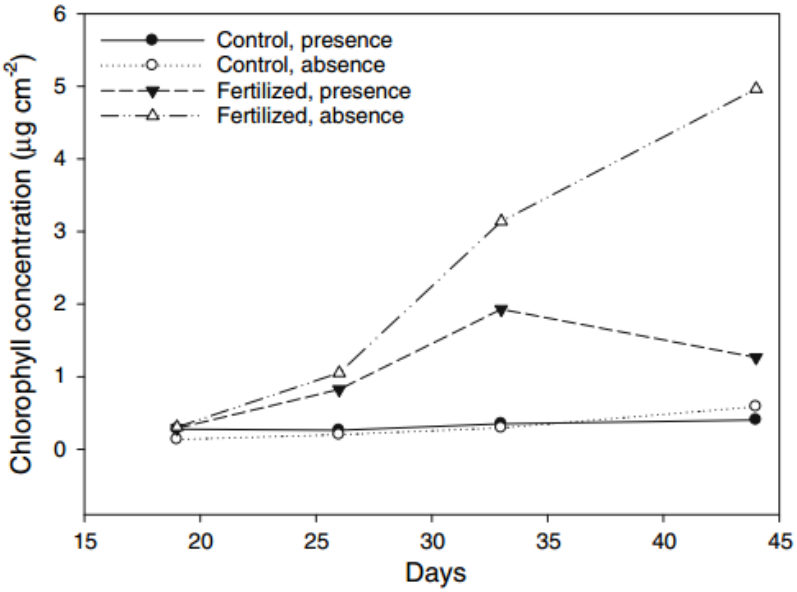


Figure 6. Concentration of detached periphyton chlorophyll *a* in fertilized and control boxes in presence and absence of grazers.

5.5. Discussion

The dissolved oxygen values were significantly higher in the fertilized than in the control reach. This change is related to the increase of the primary production as a response to the supply of nutrients (Dodds 2006), that can be assumed given the higher benthic and detached periphyton chlorophyll *a* in the fertilized reach. Dodds *et* al. (2002) also found positive correlations between the detached periphyton chlorophyll *a* and nutrients in the water column. Also, Biggs & Smith (2002) concluded that nutrients supply had strong influence on periphyton despite disturbances, e.g. resulting from hydrological stability. Both the benthic algal biomass and suspended algae increased as a result of increasing nutrient supply in Tota's stream. The nutrients concentrations reached during the experiment were within the ranges defined (Rier & Stevenson 2006 ; <86 µg DIN l^{-1} and <16 µg P l^{-1}) to sustain a maximum algal biomass growth rate.

The higher algal biomass in the fertilized reach had an impact on the nymphs biomass in the impact chambers than in the control ones. The significant higher values rise of final biomass of *Tricorythodes* nymphs in the impact when light factor was a covariable

demonstrates that the more intense grazing by the herbivores is due to the influence of light and nutrients in the growth of the periphyton, that in these situations is expressed in greater food availability for the herbivores (Larned & Santos 2000, Mosisch *et* al. 2001; Taulbee *et* al. 2005).

The decrease in the detached periphyton chlorophyll *a* concentrations suggests that *Tricorythodes* nymphs behaved also as collectors according to the descriptions given by Merrit & Cummings (1996). The larvae of many mayfly species have gathering collector feeding structures and tend to feed on the upper layers, or loosely attached, portions of the periphyton mat (Steinman 1996). This contrasts with the observations of Rivera *et* al. (2008) that considered the organisms of this family to be shedders.

The results of this experiment emphasize the ecological relevance of carrying out experiments *in situ*, where light and nutrients levels are the main restrictive factors of biomass growth in primary producers and vary naturally, than in the fixed conditions in laboratory settings. Different environmental variables can influence organisms' response. In the present experiment we showed the importance of light as a covariable factor that generates significant effects in the increase of the algal biomass and the final biomass of *Tricorythodes*.

Nutrient enhancement in the high Andean stream increased primary productivity ("bottom-up") and generated herbivores responses that regulated periphyton biomass ("top-down"). These results are in agreement with those obtained in temperate rivers (Rosemond *et* al. 1993, Hillebrand & Kahlert 2001, Liess & Hillebrand 2004, Gafner & Robinson 2007). However, enrichment in tropical systems might be even more effective than in temperate areas, where the base of the stream trophic web depends of allochthonous material and seasonality, while in the high mountain tropical streams it mostly depends on the contributions of algal biomass (Davis *et* al. 2008) and the intensity of the physical events of hydrological type (Zapata & Donato 2005).

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Chapter 6

Conclusions

6. Conclusions

Preliminar Studies on Macroinvertebrate Assemblage Distribution in an Andean Mountain Stream Neotropical (Tota, Colombia)

1. We identified 42 families of macroinvertebrates. The dominant taxa were Chironomidae, Ephemeroptera (Baetidae and Leptohyphidae), Amphipoda (Hyallelidae), and Trichoptera (Leptoceridae). Temporal variability in the discharge and altitudinal gradient in temperature, particularly in dry period are the main factors that explained the changes in macroinvertebrate assemblages.

2. Temporal differences were observed between periods with high flow and low flow. In general, the total number of families was lower during the rainy season when the density of Baetidae increases while the density of Chironomidae decreases. These changes were not as pronounced in the headwaters (Tota reach).

3. In Tota reach, Chironomidae, Baetidae, Leptohyphidae and Leptoceridae contributed to the 70 % of the total density in this site. In Cuitiva reach the same families were important, but Chironomidae had a higher abundances than in Tota. In other hand, in Iza reach, two other families were also important (Hyalellidae and Elmidae).

4. The highest dissimilarity (average dissimilarity 79.2) was found between Tota and Iza reaches and were related to higher abundances of the Trichoptera, Amphipoda and Basommatophora downstream.

5. There are differences between sand and the other habitats (rocks, leaf litter, macrophyte, and stream side). In sand the most abundant families were Chironomidae, Oligochaeta and Elmidae which contributed with near 85 % of the total density. Baetidae was the most abundant family in the rest of the habitats (except leaf litter) accompanied by Chironomidae, Hydroptilidae, Leptoceridae and Leptohyphidae in rocks and macrophytes.

6. There were not significant differences in functional feeding group and density between sites and seasons.

7. Similar results were observed with the data of the contribution of the feeding groups in each substrate. Maximum differences were found between rocks and fine sands.

Nutrient Addition Effects in a Mountain Tropical Stream (Tota, Colombia): Changes in Density, Biomass and Stoichiometry

8. Tota stream is characterized by a low nitrogen concentration and the addition favoured algae biomass measured as a chlorophyll a concentration. However, this effect was not significantly reflected in consumer density or biomass. Only a slight increase was observed in invertebrate biomass in rocks.

9. Changes between high and low discharge periods could mask other effects, as in this study the effects of nutrient addition. Low stability of sands to hydrological changes would hamper the invertebrate response to nutrients. Hydrology could also limit consumer response in rocks, in spite of the increase of periphyton biomass.

10. The Functional Feeding Groups (FFG) presented no significant differences, but was evident a slight increment in density and biomass of collector-gatherers, represented mainly by *Americobaetis* sp, *Camelobaetidus* sp and *Tharulodes* sp, species that may take advantage of detritus and periphyton availability in impact reach after the enrichment.

11. Biofilm in Tota stream showed significantly (one order of magnitude) lower C:P and N:P repect other authors. While, the C:P and N:P ratios of fishes were higher values, showing a limitation of P for top predators in Tota.

12. *Tricorythodes* sp, showed a significant decrease in CN ratio in impact reach after nutrient enrichment.

Food Web of a Tropical High Mountain Stream: Effects of Nutrient Addition

13. The $\delta^{15}N$ signal establish three trophic levels in the studied reaches along the experiment: 1) basal level with CPOM and biofilm, 2) primary consumers (macroinvertebrates: collectors-gatherers: *Heterelmis* sp, *Thraulodes* sp and *Trichorythodes* sp; and collector-filterers: *Simulium* sp, 3) predators (fishes, *Onchorhynchus mykiss* and *Trichomyterus bogotensis.*

14. Biofilm shows a higher $\delta^{15}N$ signal (between 3.5 and 4 ‰) than CPOM, except for the treatment reach after the nutrient addition where the $\delta^{15}N$ was 0.10 ‰. The average fractionation of nitrogen of the primary consumers respect to CPOM is 4,7‰ (minimum 3,8 and maximum 5,5 ‰) and respect to biofilm 1,7‰. . Predators increment in 5,9‰ (from 4,3 to 10,17‰) its $\delta^{15}N$ signal respect to primary consumers.

15. There is a strong relationship between collector-gatherers and biofilm, indicating strong reliance on algal carbon by this feeding group. *Tricorythodes* sp was the collector-gatherer that has the closest connexion with the biofilm observed with the common $\delta^{15}N$ depletion after the enrichment.

16. An increase in $\delta^{13}C$ values of biofilm was observed in both reaches after the nutrient addition. This increase could be product of flow temporality.

17. Fine detritus (FD) is the most abundant food in the four invertebrate gut contents analyzed.

18. *Oncorhychus mykiss* and *Trichomycterus bogotensis* feed on macroinvertebrates in both reaches, and in Impact reach *Oncorhychus mykiss* becomes a potential prey of *Trichomycterus bogotensis* showing that there are differences in habitat conditions that may change predatory behavior.

Effects of Eutrophication on the Interaction Between Algae and Grazers in an Andean Stream

19. The dissolved oxygen values were significantly higher in the fertilized than in the control reach. This change is related to the increase of the primary production as a response to the supply of nutrients, that can be assumed given the higher benthic and detached periphyton chlorophyll *a* in the fertilized reach**.**

20. *Tricorythodes* length was related to biomass with the equation: ln DM = -7.45 + 4.06 ln(L), where *a* and *b* are the regression constants, DM dry mass (mg) and L total body length (mm).

21. Significantly higher concentrations of benthic and detached periphyton chlorophyll *a* were observed because of fertilization.

22. When the mean light value measured in each box was used as covariable differences factor that generates significant effects in the increase of the algal biomass and the final biomass of *Tricorythodes* sp. became statistically significant. Differences were also significant when biomasses between sampling days were compared because of the rise of the nymphs final biomass in the fertilized reach in relation to the control reach.

23. The detached periphyton chlorophyll *a* was significantly higher in the chambers with mayfly nymphs. We also observed that detached periphyton chlorophyll *a* concentrations were higher in the impact than in control treatments. In the presence of mayfly nymphs the concentration of detached periphyton chlorophyll *a* concentrations were lower than in the treatments without the herbivore.